

# *Escherichia coli* Harboring *mcr-1* and *bla*<sub>CTX-M</sub> on a Novel IncF Plasmid: First Report of *mcr-1* in the United States

Patrick McGann,<sup>a</sup> Erik Snesrud,<sup>a</sup> Rosslyn Maybank,<sup>a</sup> Brendan Corey,<sup>a</sup> Ana C. Ong,<sup>a</sup> Robert Clifford,<sup>a</sup> Mary Hinkle,<sup>a</sup> Timothy Whitman,<sup>b</sup> Emil Lesho,<sup>a</sup> Kurt E. Schaecher<sup>c</sup>

Multidrug-resistant Organism Repository and Surveillance Network, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA<sup>a</sup>; Department of Infectious Diseases, Walter Reed National Military Medical Center, Bethesda, Maryland, USA<sup>b</sup>; Department of Pathology, Walter Reed National Military Medical Center, Bethesda, Maryland, USA<sup>c</sup>

The recent discovery of a plasmid-borne colistin resistance gene, *mcr-1*, in China heralds the emergence of truly pan-drug-resistant bacteria (1). The gene has been found primarily in *Escherichia coli* but has also been identified in other members of the *Enterobacteriaceae* in human, animal, food, and environmental samples on every continent (2–5). In response to this threat, starting in May 2016, all extended-spectrum-β-lactamase (ESBL)-producing *E. coli* clinical isolates submitted to the clinical microbiology laboratory at the Walter Reed National Military Medical Center (WRNMMC) have been tested for resistance to colistin by Etest. Here we report the presence of *mcr-1* in an *E. coli* strain cultured from a patient with a urinary tract infection (UTI) in the United States. The strain was resistant to colistin, but it remained susceptible to several other agents, including amikacin, piperacillin-tazobactam, all carbapenems, and nitrofurantoin (Table 1).

*E. coli* MRSN 388634 was cultured from the urine of a 49-year-old female who presented to a clinic in Pennsylvania on 26 April 2016 with symptoms indicative of a UTI. The isolate was forwarded to WRNMMC, where susceptibility testing indicated an ESBL phenotype (Table 1). The isolate was included in the first 6 ESBL-producing *E. coli* isolates selected for colistin susceptibility testing, and it was the only isolate to have a MIC of colistin of 4 μg/ml (all of the others had MICs of ≤0.25 μg/ml). The colistin MIC was confirmed by broth

TABLE 1 Antibiotic resistance profile of MRSN 388634

Antibiotic(s)	MIC(s) (μg/ml) <sup>a</sup>
Amikacin	≤8, S
Amoxicillin/clavulanate	16/8, I
Ampicillin	>16, R
Aztreonam	>16, R
Cefazolin	>16, R
Cefepime	>16, R
Ceftazidime	>16, R
Ceftriaxone	>32, R
Ciprofloxacin	>2, R
Colistin	4, R
Ertapenem	≤0.25, S
Gentamicin	>8, R
Imipenem	≤0.25, S
Levofloxacin	>4, R
Meropenem	≤0.25, S
Nitrofurantoin	≤16, S
Piperacillin-tazobactam	4/4, S
Tetracycline	>8, R
Tobramycin	>8, R
Trimethoprim-sulfamethoxazole	>2/38, R

<sup>a</sup> MICs were determined using BD Phoenix (BD Diagnostics Systems, Hunt Valley, MD, USA) with panels NMIC/ID 133, except for colistin, for which determinations were performed using Etest and manual broth microdilution; both gave MICs of colistin of 4 μg/ml. R = resistant, I = intermediate, and S = susceptible, based on CLSI guidelines (except for colistin, where EUCAST breakpoints are used).

TABLE 2 Characteristics of plasmids in *E. coli* MRSN 388634

Plasmid name	Size (kb)	Inc <sup>a</sup>	Copy no. <sup>b</sup>	Antibiotic resistance genes <sup>c</sup>
pMR0516mcr	225.7	F18:A-B1	2	<i>strA</i> , <i>strB</i> , <i>bla</i> <sub>CTX-M-55</sub> , <i>bla</i> <sub>TEM-1B</sub> , <b><i>mcr-1</i></b> , <i>sul2</i> , <i>tet(A)</i> , <i>dfrA14</i>
pMR0416ctx	47	N	1	<i>aac(3)-IVa</i> , <i>aph(4)-Ia</i> , <i>bla</i> <sub>CTX-M-14</sub> , <i>fosA3</i> , <i>mph(A)</i> , <i>floR</i> , <i>sul2</i>

<sup>a</sup> Data represent plasmid incompatibility (Inc) group designations, as determined by Plasmid Finder version 1.2 (10).

<sup>b</sup> Data represent average numbers of copies per cell, normalized to the chromosomal read coverage.

<sup>c</sup> The gene of interest is indicated in bold.

microdilution, and *mcr-1* was detected by real-time PCR (6). Whole-genome sequencing (WGS) of MRSN 388634 was performed using a PacBio RS II system and a MiSeq benchtop sequencer.

*E. coli* MRSN 388634 belonged to sequence type 457 (ST457), a rare *E. coli* ST first identified in 2008 from a urine culture in the United Kingdom (7). It was subsequently identified from a blood-stream culture in Italy, where it was found to harbor the carbapenemase genes *bla*<sub>KPC-3</sub> and *bla*<sub>CTX-M-55</sub> (8). MRSN 388634 carried 15 antibiotic resistance genes, which were harbored on two plasmids, but no carbapenemases (Table 2).

The first plasmid, pMR0516mcr, was 225,707 bp in size and belonged to incompatibility group F18:A-B1 (9). BLAST analysis indicated that pMR0516mcr represented a novel IncF plasmid. Notably, it shares 89 kb of homologous sequence with pHNSHP45-2, a *mcr-1*-carrying IncHI2 plasmid described by Liu and colleagues (1). This shared sequence contains *mcr-1* in association with *ISApI1* (1), but in pMR0516mcr it is in a different location and orientation (Fig. 1). pMR0516mcr also carried 7 additional antibiotic resistance genes, including the ESBL gene *bla*<sub>CTX-M-55</sub> (Table 2). The second plasmid, pMR0416ctx, was ~47 kb in size and was assigned to IncN (Table 2). It carried 7 antibiotic resistance genes, including *bla*<sub>CTX-M-14</sub>. A complete description of both plasmids is under preparation.

Accepted manuscript posted online 26 May 2016

Citation McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R, Hinkle M, Whitman T, Lesho E, Schaecher KE. 2016. *Escherichia coli* harboring *mcr-1* and *bla*<sub>CTX-M</sub> on a novel IncF plasmid: first report of *mcr-1* in the United States. *Antimicrob Agents Chemother* 60:4420–4421. doi:10.1128/AAC.01103-16.

Address correspondence to Patrick McGann, patrick.t.mcgann4.civ@mail.mil, or Kurt E. Schaecher, kurt.e.schaecher.mil@mail.mil.

P.M. and E.S. contributed equally to this article.

Copyright © 2016 McGann et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

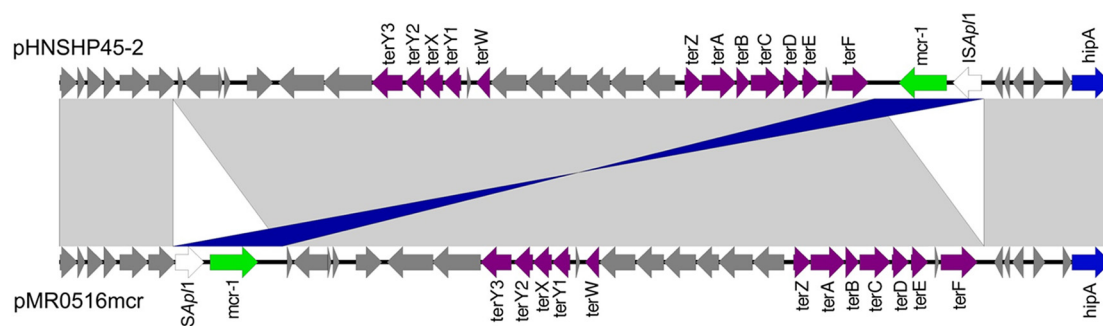


FIG 1 Comparison of the homologous regions containing *mcr-1* shared by pMR0516mcr and pHNSHP45-2. Open arrows represent coding sequences (green arrows, *mcr-1*; white arrows, *ISAp11*; purple arrows, metabolic function; blue arrows, plasmid replication and maintenance; gray arrows, hypothetical and unclassified) and indicate direction of transcription. The arrow size is proportional to the gene length. The gray and blue areas between pMR0516mcr and pHNSHP45-2 indicate nucleotide identity of >99.9% by BLASTN.

To the best of our knowledge, this is the first report of *mcr-1* in the United States. The epidemiology of MRSN 388634 is noteworthy; the isolate was submitted from a clinic in Pennsylvania, and the patient reported no travel history within the prior 5 months. To date, a further 20 ESBL-producing *E. coli* isolates from patients at the WRNMMC have tested negative for *mcr-1* and have been colistin sensitive. However, as testing has been ongoing for only 3 weeks, it remains unclear what the true prevalence of *mcr-1* is in the population. The association between *mcr-1* and IncF plasmids is concerning, as these plasmids are vehicles for the dissemination of antibiotic resistance and virulence genes among the *Enterobacteriaceae* (9). Continued surveillance to determine the true frequency for this gene in the United States is critical.

**Nucleotide sequence accession numbers.** The Short Read Archive (SRA) file for MRSN 388623 has been deposited at GenBank with accession number [SRP075674](https://www.ncbi.nlm.nih.gov/SRA/entry/1000000000). The complete sequence of pMR0516mcr has been deposited at GenBank with accession no. [KX276657](https://www.ncbi.nlm.nih.gov/GenBank/entry/1000000000).

## ACKNOWLEDGMENTS

This study was funded by the U.S. Army Medical Command, the Global Emerging Infections Surveillance and Response System, and the Defense Medical Research and Development Program. This project was performed as part of a quality improvement and infection control initiative authorized by policy no. 15-042.

We declare that we have no conflicts of interest.

The identification of specific products or scientific instrumentation does not constitute endorsement or implied endorsement on the part of the authors, the Department of Defense (DoD), or any component agency. While we generally excise references to products, companies, manufacturers, organizations, etc., in government-produced works, the abstracts produced and other similarly situated research present a special circumstance when such product inclusions become an integral part of the scientific endeavor.

The material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation.

The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Army/Navy/Air Force, Department of Defense, or the U.S. Government.

## FUNDING INFORMATION

This work, including the efforts of Emil Lesho, was funded by U.S. Army Medical Command (MedCom 15-042). This work, including the efforts of Emil Lesho, was funded by Global Emerging Infections Surveillance (20160280023).

## REFERENCES

- Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D,

- Zhou H, Liang Z, Liu JH, Shen J. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 16:161–168. [http://dx.doi.org/10.1016/S1473-3099\(15\)00424-7](http://dx.doi.org/10.1016/S1473-3099(15)00424-7).
- Fernandes MR, Moura Q, Sartori L, Silva KC, Cunha MP, Esposito F, Lopes R, Otutumi LK, Goncalves DD, Dropa M, Matte MH, Monte DF, Landgraf M, Francisco GR, Bueno MF, de Oliveira Garcia D, Knobl T, Moreno AM, Lincopan N. 28 April 2016. Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr-1* gene. *Euro Surveill* <http://dx.doi.org/10.2807/1560-7917.ES.2016.21.17.30214>.
- Rapoport M, Faccione D, Pasteran F, Ceriana P, Alborno E, Petroni A; MCR-Group, Corso A. 18 April 2016. *mcr-1*-mediated colistin resistance in human infections caused by *Escherichia coli*: first description in Latin America. *Antimicrob Agents Chemother* <http://dx.doi.org/10.1128/AAC.00573-16>.
- Skov RL, Monnet DL. 2016. Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. *Euro Surveill* <http://dx.doi.org/10.2807/1560-7917.ES.2016.21.9.30155>.
- Zeng KJ, Doi Y, Patil S, Huang X, Tian GB. 23 May 2016. Emergence of plasmid-mediated *mcr-1* gene in colistin-resistant *Enterobacter aerogenes* and *Enterobacter cloacae*. *Antimicrob Agents Chemother* <http://dx.doi.org/10.1128/AAC.00345-16>.
- Bontron S, Poirel L, Nordmann P. 27 April 2016. Real-time PCR for detection of plasmid-mediated polymyxin resistance (*mcr-1*) from cultured bacteria and stools. *J Antimicrob Chemother* <http://dx.doi.org/10.1093/jac/dkw139>.
- Lau SH, Reddy S, Cheesbrough J, Bolton FJ, Willshaw G, Cheasty T, Fox AJ, Upton M. 2008. Major uropathogenic *Escherichia coli* strain isolated in the northwest of England identified by multilocus sequence typing. *J Clin Microbiol* 46:1076–1080. <http://dx.doi.org/10.1128/JCM.02065-07>.
- Accogli M, Giani T, Monaco M, Giufre M, Garcia-Fernandez A, Conte V, D'Ancona F, Pantosti A, Rossolini GM, Cerquetti M. 2014. Emergence of *Escherichia coli* ST131 sub-clone H30 producing VIM-1 and KPC-3 carbapenemases, Italy. *J Antimicrob Chemother* 69:2293–2296. <http://dx.doi.org/10.1093/jac/dku132>.
- Villa L, Garcia-Fernandez A, Fortini D, Carattoli A. 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. *J Antimicrob Chemother* 65:2518–2529. <http://dx.doi.org/10.1093/jac/dkq347>.
- Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 58:3895–3903. <http://dx.doi.org/10.1128/AAC.02412-14>.

## ERRATUM

**Erratum for McGann et al., *Escherichia coli* Harboring *mcr-1* and *bla*<sub>CTX-M</sub> on a Novel IncF Plasmid: First Report of *mcr-1* in the United States**

Patrick McGann,<sup>a</sup> Erik Snesrud,<sup>a</sup> Rosslyn Maybank,<sup>a</sup> Brendan Corey,<sup>a</sup> Ana C. Ong,<sup>a</sup> Robert Clifford,<sup>a</sup> Mary Hinkle,<sup>a</sup> Timothy Whitman,<sup>b</sup> Emil Lesho,<sup>a</sup> Kurt E. Schaecher<sup>c</sup>

Multidrug-resistant Organism Repository and Surveillance Network, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA<sup>a</sup>; Department of Infectious Diseases, Walter Reed National Military Medical Center, Bethesda, Maryland, USA<sup>b</sup>; Department of Pathology, Walter Reed National Military Medical Center, Bethesda, Maryland, USA<sup>c</sup>

Volume 60, no. 7, p. 4420–4421, 2016. Page 4420, right column, line 15: the correct reference for the sequence and description of plasmid pHNSHP45-2 is not reference 1 but is as follows.

Zhi C, Lv L, Yu L-F, Doi Y, Liu J-H. 2016. Dissemination of the *mcr-1* colistin resistance gene. *Lancet Infect Dis* 16:292-293. [http://dx.doi.org/10.1016/S1473-3099\(16\)00063-3](http://dx.doi.org/10.1016/S1473-3099(16)00063-3).

**Citation** McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R, Hinkle M, Whitman T, Lesho E, Schaecher KE. 2016. Erratum for McGann et al., *Escherichia coli* harboring *mcr-1* and *bla*<sub>CTX-M</sub> on a novel IncF plasmid: first report of *mcr-1* in the United States. *Antimicrob Agents Chemother* 60:5107. doi:10.1128/AAC.01353-16.  
Copyright © 2016, American Society for Microbiology. All Rights Reserved.