

CNA<sup>+</sup> medium did not identify intestinal carriage of ESBL-negative but *mcr-1*-positive enterobacteria in the index case-patient. On the basis of these results, rectal screening of 39 contacts was performed by using an ESBL-screening medium (BLSE agar [MacConkey agar and Drigalski agar]; bioMérieux). All of the tests showed negative results.

The origin of the *mcr-1* strain remains unknown. Nosocomial acquisition cannot be ruled out because colistin-resistant strains harboring the *mcr-1* gene might have been isolated in the hospital but not identified because this resistance mechanism was initially reported in February 2016. Food might also be incriminated (1); one study identified a 21% *mcr-1* prevalence among ESBL-producing *E. coli* in calves in France (10).

Multiple antimicrobial drug therapy for this patient might have selected for this multidrug-resistant bacteria. The presence of a plasmid containing the *mcr-1* and ESBL or other resistance genes in the same strain might be involved in selection of colistin-resistant strains during administration of any ineffective antimicrobial drug (3). Development of efficient tools for rapid detection of *mcr-1*-harboring strains should be a priority to prevent dissemination of these strains in hospital settings.

Dr. Caspar is a clinical microbiologist in the Bacteriology Laboratory, Grenoble-Alpes University Hospital, and a probationary lecturer in the Medicine Faculty, Grenoble-Alpes University, Grenoble, France. His primary research interests are bacterial resistance or treatment failures for tularemia and evaluation of novel antimicrobial compounds.

## References

1. Baron S, Hadjadj L, Rolain J-M, Olaitan AO. Molecular mechanisms of polymyxin resistance: knowns and unknowns. *Int J Antimicrob Agents*. 2016;48:583–91. <http://dx.doi.org/10.1016/j.ijantimicag.2016.06.023>
2. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, et al. 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*. 2016;16:161–8. [http://dx.doi.org/10.1016/S1473-3099\(15\)00424-7](http://dx.doi.org/10.1016/S1473-3099(15)00424-7)
3. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. *J Antimicrob Chemother*. 2016;71:2066–70. <http://dx.doi.org/10.1093/jac/dkw274>
4. Di Pilato V, Arena F, Tascini C, Cannatelli A, Henrici De Angelis L, Fortunato S, et al. *mcr-1.2*, a new *mcr* variant carried on a transferable plasmid from a colistin-resistant KPC carbapenemase-producing *Klebsiella pneumoniae* strain of sequence type 512. *Antimicrob Agents Chemother*. 2016;60:5612–5. <http://dx.doi.org/10.1128/AAC.01075-16>
5. Cannatelli A, D'Andrea MM, Giani T, Di Pilato V, Arena F, Ambretti S, et al. *In vivo* emergence of colistin resistance in *Klebsiella pneumoniae* producing KPC-type carbapenemases mediated by insertional inactivation of the PhoQ/PhoP *mgrB* regulator. *Antimicrob Agents Chemother*. 2013;57:5521–6. <http://dx.doi.org/10.1128/AAC.01480-13>
6. Nordmann P, Jayol A, Poirel L. A universal culture medium for screening polymyxin-resistant gram-negative isolates. *J Clin Microbiol*. 2016;54:1395–9. <http://dx.doi.org/10.1128/JCM.00446-16>
7. Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in *Enterobacteriaceae*. *Emerg Infect Dis*. 2016;22:1038–43. <http://dx.doi.org/10.3201/eid2206.151840>
8. Nijhuis RH, Veldman KT, Schelfaut J, Van Essen-Zandbergen A, Wessels E, Claas EC, et al. Detection of the plasmid-mediated colistin-resistance gene *mcr-1* in clinical isolates and stool specimens obtained from hospitalized patients using a newly developed real-time PCR assay. *J Antimicrob Chemother*. 2016;71:2344–6. <http://dx.doi.org/10.1093/jac/dkw192>
9. Bontron S, Poirel L, Nordmann P. Real-time PCR for detection of plasmid-mediated polymyxin resistance (*mcr-1*) from cultured bacteria and stools. *J Antimicrob Chemother*. 2016;71:2318–20. <http://dx.doi.org/10.1093/jac/dkw139>
10. Haenni M, Métayer V, Gay E, Madec J-Y. Increasing trends in *mcr-1* prevalence among ESBL-producing *E. coli* in French calves despite decreasing exposure to colistin. *Antimicrob Agents Chemother*. 2016;60:6433–4. <http://dx.doi.org/10.1128/AAC.01147-16>

Address for correspondence: Yvan Caspar, Laboratoire de Bactériologie, Institut de Biologie et Pathologie, Centre Hospitalier Universitaire Grenoble Alpes, CS10217, 38043 Grenoble CEDEX 9, France; email: [ycaspar@chu-grenoble.fr](mailto:ycaspar@chu-grenoble.fr)

## Chromosomal 16S Ribosomal RNA Methyltransferase RmtE1 in *Escherichia coli* Sequence Type 448

Bin Li, Marissa P. Pacey, Yohei Doi

Author affiliations: Fujian Medical University Union Hospital, Fuzhou, China (B. Li); University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA (B. Li, M.P. Pacey, Y. Doi)

DOI: <http://dx.doi.org/10.3201/eid2305.162000>

We identified *rmtE1*, an uncommon 16S ribosomal methyltransferase gene, in an aminoglycoside- and cephalosporin-resistant *Escherichia coli* sequence type 448 clinical strain co-harboring *bla*<sub>CMY-2</sub>. Long-read sequencing revealed insertion of a 101,257-bp fragment carrying both resistance genes to the chromosome. Our findings underscore *E. coli* sequence type 448 as a potential high-risk multidrug-resistant clone.

**R**mtE (RmtE1 and its variant RmtE2) is an uncommon plasmid-mediated 16S rRNA methyltransferase (16S RMTase) found in gram-negative bacteria; only 4 strains have been reported to produce RmtE, all *Escherichia coli*, including 1 from the University of Pittsburgh Medical

Center (Pittsburgh, PA, USA) (1–3). We report the genetic context of *rmtE* (*rmtE1*) in another *E. coli* clinical strain identified at this hospital.

*E. coli* YDC774 was identified in 2016 in the urine of a local elderly man with a history of bladder cancer for which he had undergone transurethral resection of the bladder and completed chemotherapy. He had *E. coli* urinary tract infection treated with ciprofloxacin 3 months earlier; further details were unavailable. *E. coli* YDC774 was resistant to cefotaxime, levofloxacin, ciprofloxacin, and trimethoprim/sulfamethoxazole and susceptible to ceftazidime, cefepime, piperacillin/tazobactam, imipenem, meropenem, minocycline, and colistin. The strain was highly resistant to amikacin (MIC >32 µg/mL), gentamicin (MIC >16 µg/mL), and tobramycin (MIC >8 µg/mL). Because the positive culture was believed to represent asymptomatic bacteriuria, the patient was not treated with antimicrobial drugs.

We aimed to elucidate the genetic context of *rmtE* in *E. coli* YDC774. Although *rmtE* has been identified exclusively on plasmids, neither broth conjugation with *E. coli* J53 nor transformation of *E. coli* TOP10 with purified plasmids mobilized *rmtE*, leading us to speculate the gene might be located on the chromosome. We therefore sequenced the YDC774 genome with PacBio RS II sequencing instrument (Pacific Biosciences, Menlo Park, CA, USA) as described (4). Sequencing with a single SMRT cell yielded 64,878 reads averaging 10,991 bp. De novo assembly generated 8 contigs; the largest was ≈4.3 Mbp, which had ≈122× coverage and was consistent with a large portion of the *E. coli* chromosome.

*E. coli* YDC774 belonged to sequence type (ST) 448 by in silico multilocus sequence typing. *E. coli* ST448 has been reported in recent years among extended-spectrum β-lactamase- and New Delhi-type metallo-β-lactamase-producing strains (5,6). The chromosomal contig contained *rmtE* (*rmtE1* allele), *bla*<sub>CMY-2</sub>, *aac(3)-VIa*, *aadA*, *strA/B*, *floR*, *sul1*, *sul2*, *tet(A)*, and *dfpA7* as determined by ResFinder (<https://cge.cbs.dtu.dk/services/ResFinder/>). We identified no resistance genes on the other contigs, including those representing a 96-kb IncY plasmid resembling p12579\_1 (GenBank accession no. CP003110.1) in enteropathogenic *E. coli* strain RM12579 (99% identity over 83% coverage). Several other 16S RMTase genes, such as *rmtB*, *rmtC*, and *rmtF*, have been found on the chromosome of gram-negative bacteria (7,8).

The region surrounding *rmtE1* was annotated with Rapid Annotations by using Subsystem Technology server (<http://rast.nmpdr.org>) and curated manually by using blastn and blastp (<http://blast.ncbi.nlm.nih.gov/blast>) to elucidate the context of its chromosomal integration. Using *E. coli* ATCC 25922 as the reference genome, we

determined that a 101,257-bp sequence was inserted in an intergenic region between the 4'-phosphopantetheinyl transferase gene and the NAD(P)H nitroreductase gene on the *E. coli* chromosome.

This inserted sequence can be divided into 2 regions. The first comprises several inserted sequences, such as *IS186*, *ISCR1*, and 1 antimicrobial resistance gene, *aadA*. Downstream of this first region, the inserted fragment is similar to that in pYDC637, an IncA/C plasmid carrying *rmtE1* also found at the University of Pittsburgh Medical Center in 2012 (online Technical Appendix Figure, <https://wwwnc.cdc.gov/EID/article/23/5/16-2000-Techapp1.pdf>) (2). However, the second region comprises 3 small fragments. The first contains *aadA1-bx*, 4 mobile elements, and several other genes and is in reverse orientation from that of pYDC637. The second small fragment harboring *bla*<sub>CMY-2</sub> is identical to that found in the core region in pYDC637 (online Technical Appendix Figure) and also is in reverse orientation from the corresponding region of pYDC637. The third small fragment harboring *rmtE* is located in the acquired region of pYDC637. This finding suggests that, on mobilization into the chromosome, gene rearrangements occurred among these fragments. The region between 2 hypothetical proteins appears to have been deleted at or after integration, which includes genes involved in plasmid replication and conjugative transfer (online Technical Appendix Figure).

*rmtE1* is bound by an *ISCR20*-like element and an *IS1294*-like insertion sequence. This immediate unit is identical to that found in pYDC637. *ISCR20* and *IS1294* belong to *IS91* family, which is considered related to some antimicrobial drug resistance genes, including 16S RMTase genes, which appears to have been the case in the mobilization of *rmtE1* as well. We could not identify direct repeats upstream and downstream of the unit that would define the exact boundary of this unit. In comparing the genetic context of *rmtE1* and *rmtE2*, *ISCR20*-like transposase is located upstream of *rmtE1* and *rmtE2* (GenBank accession nos. KT428293 and NZ\_LR1X01000127). However, the transposase genes located downstream of the 2 16S RMTase genes are distinct. The genetic environment of *rmtE2* is identical between the 2 plasmids from China (GenBank accession no. KT428293) and Canada (GenBank accession no. NZ\_LR1X01000127).

In summary, we identified chromosomal integration of *rmtE1*, an unusual 16S RMTase, and *bla*<sub>CMY-2</sub>, a commonly observed acquired AmpC β-lactamase, in an *E. coli* ST448 clinical strain, an event that generated stable co-resistance to aminoglycosides and oxyiminocephalosporins. We found no evidence of further spread of this strain in the hospital. Nonetheless, the findings underscore *E. coli* ST448 as a potential high-risk multidrug-resistant *E. coli* clone.

Y.D. was supported by research grants from the National Institutes of Health (R01AI104895, R21AI123747).

Dr. Li is a visiting researcher at the University of Pittsburgh. His research interests include mechanisms of multidrug resistance in gram-negative bacteria.

## References

1. Davis MA, Baker KN, Orfe LH, Shah DH, Besser TE, Call DR. Discovery of a gene conferring multiple-aminoglycoside resistance in *Escherichia coli*. *Antimicrob Agents Chemother*. 2010;54:2666–9. <http://dx.doi.org/10.1128/AAC.01743-09>
2. Lee CS, Li JJ, Doi Y. Complete sequence of conjugative IncA/C plasmid encoding CMY-2  $\beta$ -lactamase and RmtE 16S rRNA methyltransferase. *Antimicrob Agents Chemother*. 2015;59:4360–1. <http://dx.doi.org/10.1128/AAC.00852-15>
3. Xia J, Sun J, Li L, Fang LX, Deng H, Yang RS, et al. First report of the IncII/ST898 conjugative plasmid carrying *rmtE2* 16S rRNA methyltransferase gene in *Escherichia coli*. *Antimicrob Agents Chemother*. 2015;59:7921–2. <http://dx.doi.org/10.1128/AAC.01235-15>
4. Thomson GK, Snyder JW, McElheny CL, Thomson KS, Doi Y. Coproduction of KPC-18 and VIM-1 carbapenemases by *Enterobacter cloacae*: implications for newer  $\beta$ -lactam- $\beta$ -lactamase inhibitor combinations. *J Clin Microbiol*. 2016;54:791–4. <http://dx.doi.org/10.1128/JCM.02739-15>
5. Blaak H, Hamidjaja RA, van Hoek AH, de Heer L, de Roda Husman AM, Schets FM. Detection of extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Escherichia coli* on flies at poultry farms. *Appl Environ Microbiol*. 2014;80:239–46. <http://dx.doi.org/10.1128/AEM.02616-13>
6. Baraniak A, Izdebski R, Fielt J, Gawryszewska I, Bojarska K, Herda M, et al. NDM-producing *Enterobacteriaceae* in Poland, 2012–14: inter-regional outbreak of *Klebsiella pneumoniae* ST11 and sporadic cases. *J Antimicrob Chemother*. 2016;71:85–91. <http://dx.doi.org/10.1093/jac/dkv282>
7. Yu T, He T, Yao H, Zhang JB, Li XN, Zhang RM, et al. Prevalence of 16S rRNA methylase gene *rmtB* among *Escherichia coli* isolated from bovine mastitis in Ningxia, China. *Foodborne Pathog Dis*. 2015;12:770–7. <http://dx.doi.org/10.1089/fpd.2015.1983>
8. Rahman M, Prasad KN, Pathak A, Pati BK, Singh A, Ovejero CM, et al. RmtC and RmtF 16S rRNA methyltransferase in NDM-1-producing *Pseudomonas aeruginosa*. *Emerg Infect Dis*. 2015;21:2059–62. <http://dx.doi.org/10.3201/eid2111.150271>

Address for correspondence: Yohei Doi, Division of Infectious Diseases, University of Pittsburgh School of Medicine, S829 Scaife Hall, 3550 Terrace St, Pittsburgh, PA 15261, USA; email: yod4@pitt.edu

# Carbapenem-Resistant *Enterobacter cloacae* in Patients from the US Veterans Health Administration, 2006–2015

Brigid M. Wilson, Nadim G. El Chakhtoura, Sachin Patel, Elie Saade, Curtis J. Donskey, Robert A. Bonomo, Federico Perez

Author affiliations: Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio, USA (B.M. Wilson, N.G. El Chakhtoura, C.J. Donskey, R.A. Bonomo, F. Perez); University Hospitals Cleveland Medical Center, Cleveland (N.G. El Chakhtoura, S. Patel, E. Saade, R.A. Bonomo); Case Western Reserve University School of Medicine, Cleveland (C.J. Donskey, R.A. Bonomo, F. Perez)

DOI: <http://dx.doi.org/10.3201/eid2305.162034>

We analyzed carbapenem-resistant *Enterobacteriaceae* (CRE) trends among patients from the US Veterans Health Administration (VHA). After the emergence of CRE in the eastern United States, resistance rates remained stable in *Klebsiella pneumoniae* but increased in *Enterobacter cloacae* complex, suggesting a “second epidemic.” VHA offers a vantage point for monitoring nationwide CRE trends.

Carbapenem-resistant *Enterobacteriaceae* (CRE) have become a global public health threat. The epidemic of CRE began in the early 2000s with an outbreak of carbapenem-resistant *Klebsiella pneumoniae* harboring *K. pneumoniae* carbapenemase (KPC) in the eastern United States. Since then, KPC-producing *K. pneumoniae* have emerged in various communities across the country (1). Carbapenem resistance also occurs in other *Enterobacteriaceae* species and can be mediated by other enzymes, such as OXA-48 and metallo- $\beta$ -lactamases, especially New Delhi metallo- $\beta$ -lactamase and Verona integron-encoded metallo- $\beta$ -lactamase (2). Carbapenem-resistant *Escherichia coli* occurs infrequently, but recent outbreaks of KPC-producing *Enterobacter cloacae* raise concerns about the emergence of carbapenem resistance in the *E. cloacae* complex (3–4).

The Veterans Health Administration (VHA) is the largest integrated healthcare system in the United States. Clinical and microbiologic data for the entire VHA network are accessible through its informatics platforms (5). We used this infrastructure to observe national trends of carbapenem resistance and nonsusceptibility in *K. pneumoniae* and *E. cloacae* complex during the past decade.

We identified 224,651 *K. pneumoniae* and 71,462 *E. cloacae* complex (*E. cloacae*, *E. asburiae*, *E. kobei*,

