AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EPIDEMIOLOGY AND SURVEILLANCE

Escherichia coli Harboring *mcr-1* in a Cluster of Liver Transplant Recipients: Detection through Active Surveillance and Whole-Genome Sequencing

Nenad Macesic,[a,b] Sabrina Khan,[a] Marla J. Giddins,[a] Daniel E. Freedberg,[c] Susan Whittier,[d] Daniel A. Green,[d] E. Yoko Furuya,[a] Elizabeth C. Verna,[c] Medini K. Annavajhala,[a] Angela Gomez-Simmonds,[a] Anne-Catrin Uhlemann[a]

[a]Division of Infectious Diseases, Columbia University Irving Medical Center, New York, New York, USA
[b]Central Clinical School, Monash University, Melbourne, Victoria, Australia
[c]Division of Digestive and Liver Diseases, Columbia University Irving Medical Center, New York, New York, USA
[d]Clinical Microbiology Laboratory Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, New York, USA

ABSTRACT *mcr-1*, a plasmid-associated gene for colistin resistance, was first described in China in 2015, but its spread in the United States is unknown. We report detection of *mcr-1*-carrying *Escherichia coli* ST117 in a cluster of three liver transplant recipients.

KEYWORDS antimicrobial resistance, colistin, *mcr-1*, polymyxin B, whole-genome sequencing

Polymyxins are treatments of "last resort" for multidrug-resistant Gram-negative bacterial (MDR-GNB) infections. *mcr-1*, a plasmid-associated gene for polymyxin resistance, was first described in China in 2015, with widespread dissemination noted (1, 2), but only 53 U.S. cases have been reported to date (https://www.cdc.gov/drugresistance/biggest-threats/tracking/mcr.html). We report a cluster of *Escherichia coli* sequence type (ST) 117 harboring *mcr-1* in three liver transplant recipients after an initial case of infection in 2015 (3).

The index case (patient 1) had bloodstream and biliary fluid infection with *mcr-1*-carrying *E. coli* (3). The remaining three patients carried *mcr-1*-positive *E. coli* in stool samples collected during the prospective liver transplant (PLT) study but did not experience infections (4). We conducted an epidemiological analysis in order to discern possible transmission events (Fig. 1).

Patient 1 underwent elective biliary tree excision and Roux-en-Y hepaticojejunostomy in May 2015 for recurrent episodes of cholangitis on a background of sickle-cell anemia and cholecystectomy. Intraoperative biliary cultures grew *mcr-1*-positive *E. coli*. The patient became febrile the day after surgery, and blood cultures also grew *mcr-1*-positive *E. coli*. The patient was successfully treated with meropenem. Patients 2 to 4 had undergone liver transplant: two patients in November 2014 and one in January 2015. Patients 2 to 4 regularly provided fecal samples as part of the PLT study (Fig. 1) and were noted to have new onset MDR *E. coli* colonization at days 241, 275, and 322 posttransplant, respectively, prompting WGS and retrospective detection of *mcr-1*.

Patient 2 underwent same-day endoscopic retrograde cholangiopancreatography (ERCP), as did patient 1, in March 2015, and the first available subsequent stool sample was positive for *mcr-1* 6 months later (Fig. 1). Patient 3 underwent gastroduodenoscopy the same day patient 2 underwent ERCP in September 2015. Of note, patient 3 had a stool sample negative for *mcr-1* 6 days prior to the endoscopy, followed by detection of *mcr-1* 7 days following the endoscopy, suggesting acquisition around the time of endoscopy. Patient 4 had no obvious epidemiologic links to explain *mcr-1* acquisition.

Citation Macesic N, Khan S, Giddins MJ, Freedberg DE, Whittier S, Green DA, Furuya EY, Verna EC, Annavajhala MK, Gomez-Simmonds A, Uhlemann A-C. 2019. *Escherichia coli* harboring *mcr-1* in a cluster of liver transplant recipients: detection through active surveillance and whole-genome sequencing. Antimicrob Agents Chemother 63:e02680-18. https://doi.org/10.1128/AAC.02680-18.

Address correspondence to Nenad Macesic, nm2891@cumc.columbia.edu, or Anne-Catrin Uhlemann, au2110@columbia.edu.

Received 27 December 2018
Returned for modification 27 January 2019
Accepted 19 March 2019
Accepted manuscript posted online 8 April 2019
Published 24 May 2019

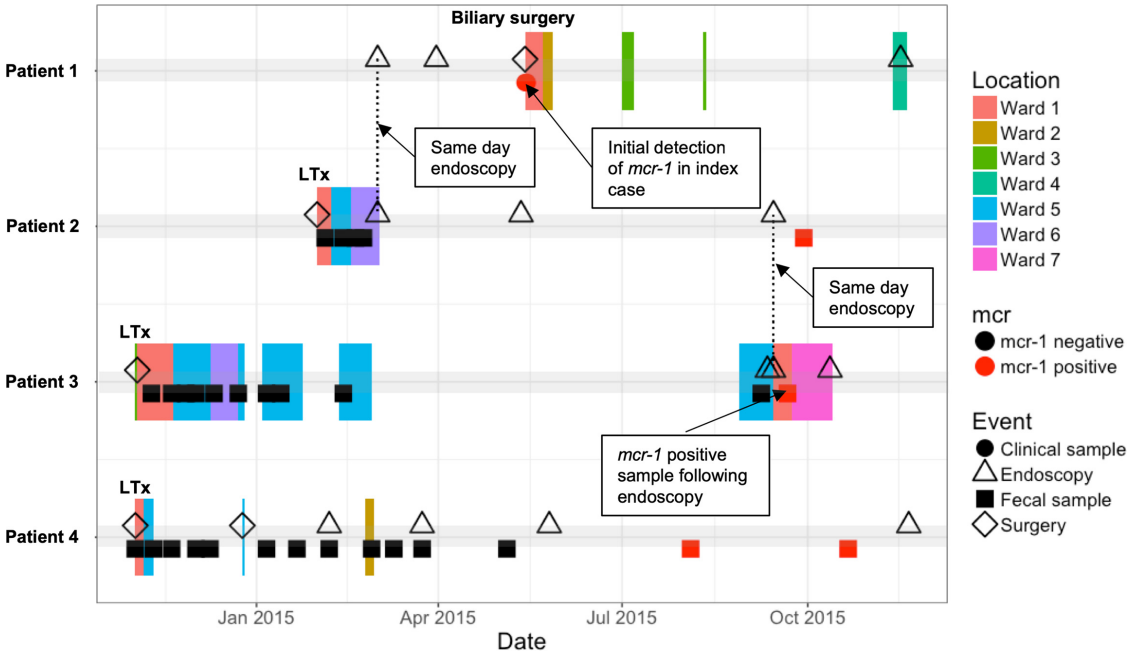

FIG 1 Epidemiological analysis of cluster of patients colonized and infected with health care-associated *mcr-1*-carrying *E. coli*. Patient 1 was the index case and had infection with *mcr-1*-carrying *E. coli* in biliary fluid and blood isolates following elective biliary surgery. Subsequently, closely related *mcr-1*-carrying *E. coli* was detected in fecal samples of patients 2 to 4, who had all undergone liver transplantation. Endoscopy performed on the same day appeared to be a common epidemiological link for patients 1, 2, and 4 (indicated with dotted lines). No such link was found for patient 3. LTx, liver transplant.

No overlap was noted between this patient and other cluster patients with respect to endoscopy procedures, outpatient visits, or radiology investigations. The patients all lived in different zip codes. During 2.5 years of follow-up, patients 2 to 4 did not develop any *mcr-1*-associated infections.

Multilocus sequence typing indicated that all isolates were ST117. Patients 1 and 4 had two isolates each available for analysis, while patients 2 and 3 had single isolates. Cultured isolates underwent identification and susceptibility testing with Vitek-2 (bioMérieux). We performed MIC determination for colistin and polymyxin B with broth microdilution according to established guidelines (5). Tigecycline susceptibility testing was performed with Etest (bioMérieux). Details of whole-genome sequencing (WGS) are provided in the supplemental material.

The following MIC ranges were noted on a Vitek-2 instrument (bioMérieux, Durham, NC): ceftriaxone, 2 to 8 μg/ml; cefepime, $<$1 μg/ml; aztreonam, $>$64 μg/ml; ertapenem, $<$0.5 μg/ml; meropenem, $<$0.25 μg/ml; amikacin, $<$2 μg/ml; tobramycin, $<$1 μg/ml; gentamicin, $<$1 μg/ml; levofloxacin, $>$8 μg/ml; and tigecycline, 0.38 to 0.75 μg/ml. In addition to *mcr-1*, isolates harbored resistance genes to beta-lactams (bla_{SHV-12} and bla_{TEM-1D}), aminoglycosides [*aph(3′)-IIa*, *aph(6)-Ic*, *aadA2*, *aph(3′)-Ia*, *strA*, and *strB*], sulfonamides (*sul1* and *sul2*), trimethoprim (*dfrA12*), chloramphenicol (*catA1*), macrolides (*mphA*), and tetracycline [*tet*(*A*)]. Of note, bla_{SHV-12} codes for an extended-spectrum beta-lactamase (6). Full isolate antimicrobial susceptibilities, resistance genes, and plasmid replicons are shown in Tables S2 to S4 in the supplemental material. Within-patient pairwise single nucleotide variant (SNV) distances of patient 1 and patient 4 isolates were 15 and 3 SNV (Table S1). Pairwise SNV distances of isolates from different patients ranged from 7 to 28 SNVs. Phylogenetic relationships are shown in Fig. 2. The *mcr-1* gene was carried on an IncX4 plasmid in all isolates. The plasmid replicon content was similar for the isolates.

We then conducted a retrospective molecular survey using *mcr-1* PCR to evaluate for possible spread of *mcr-1* (1). First, we tested all 197 *E. coli* isolates resistant to third-generation cephalosporins cultured from PLT study patients from 2015 to 2017.

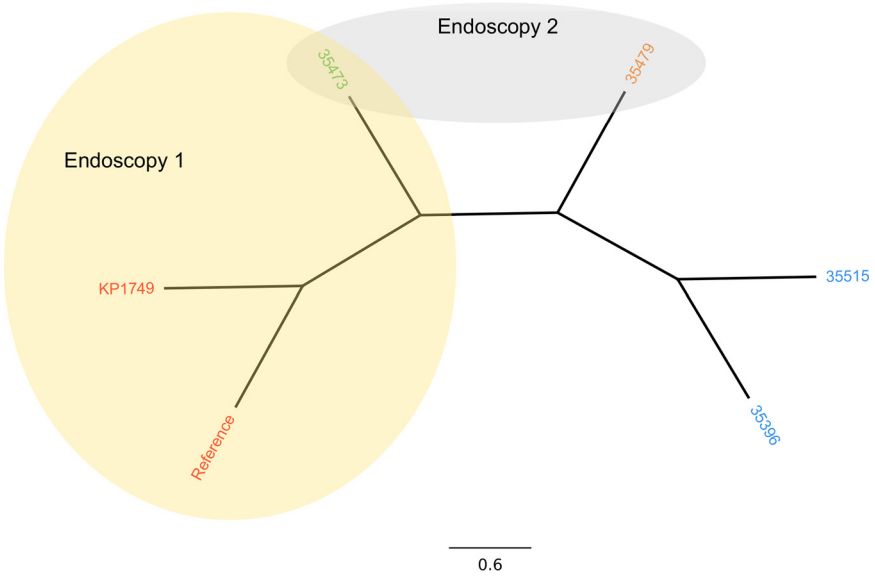

FIG 2 Phylogenetic tree of *mcr-1*-carrying *E. coli* isolates. The genome of isolate NR2148 (GenBank accession no. CP019903.1) was the reference. Different patients are represented by colored nodes (red, patient 1; green, patient 2; orange, patient 3; blue, patient 4). The epidemiological associations for the shared endoscopy location are indicated by the colored fields.

We then tested 187 discarded surveillance rectal swabs of consecutive patients admitted to medical and surgical intensive care units (ICUs) in 2017. Finally, we tested 123 stored clinical *E. coli* isolates resistant to third-generation cephalosporins collected from 2011 to 2016. No further cases of *mcr-1*-carrying isolates were detected ($n = 507$).

The detection of this cluster demonstrates the potential for silent dissemination of *mcr-1* in a U.S. hospital setting through asymptomatic colonization and suggests a possible role for same-day endoscopy-related transmission, independent of using the same endoscope. This represents the earliest documented health care-associated cluster of *mcr-1* in the United States and predates a recent report from 2017 (7). Both reports implicate endoscopy as a potential route for transmission. The current report also highlights the difficulty of instituting surveillance measures for *mcr-1*-harboring isolates when endemicity is low. While infective episodes have the greatest impact on patient outcomes, detection of silent colonization may play an important role in stemming the spread of *mcr-1*.

Our epidemiological analysis showed the possible role of endoscopy, with three patients having same-day endoscopies. However, the same endoscopes were not used during same-day procedures. Other mechanisms, such as a common environmental source, may have contributed. A previous outbreak of KPC-2 *Klebsiella pneumoniae* implicated a positioning pillow (8). Although endoscopy location was the clearest association, there were multiple other possible contributors since these patients had frequent radiological investigations, were cared for by the same caregivers, and had admissions to the same hospital wards. This may explain the acquisition in patient 4. Unfortunately, *mcr-1* was only detected several years after sample collection, limiting our ability to conduct a real-time investigation. Our isolate collections also focused only on *E. coli* isolates resistant to third-generation cephalosporins. While *mcr-1* colonization was found in these patients due to participation in a prospective study, colonization in other patients may have gone undetected. Nevertheless, our molecular survey of over 500 samples did not detect additional cases, either before or since (through 2017), demonstrating that *mcr-1* has not yet become widespread. Furthermore, colonization in all three liver transplant recipients was not associated with clinical infection.

Genomic analysis confirmed that isolates belonged to ST117, were related, and carried *mcr-1* on near-identical IncX4 plasmids. The distance of 15 SNVs between

isolates from the index patient was high and suggests possible within-host evolution through long-term colonization. This relatively high SNV distance has direct implications for defining related clusters and spread. Conversely, the reference isolate was less distant from isolate 35479 from patient 2 (7 SNVs), suggesting transmission.

Our findings highlight the need for ongoing surveillance of *mcr-1* and other forms of transferrable resistance to polymyxins in the United States. Our analysis suggested endoscopy location as an epidemiologic link but did not implicate endoscopes directly. Although we documented acquisition of *mcr-1* colonization in several liver transplant recipients through active surveillance of fecal MDR-GNB carriage and WGS, this is a highly resource-intensive approach and is not applicable on a larger scale. A more viable alternative may be to implement PCR screening of discarded specimens (e.g., stool samples or rectal swabs) (9). In order to stop further spread of emerging forms of polymyxin resistance, future surveillance approaches need to recognize that clinical isolates may only represent the "tip of the iceberg" formed by the burden of asymptomatic colonization.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02680-18.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

This study was supported by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (grants AI116939 to A.-C.U. and S1-AI116939 to A.-C.U. and A.G.-S.) and by an Irving Scholarship from Columbia University (A.-C.U.).

All authors report no conflicts of interest relevant to the manuscript.

REFERENCES

1. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu L-F, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu J-H, 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. https://doi.org/10.1016/S1473-3099(15)00424-7.
2. Zhong LL, Phan HTT, Shen C, Doris-Vihta K, Sheppard AE, Huang X, Zeng KJ, Li HY, Zhang XF, Patil S, Crook DW, Walker AS, Xing Y, Lin JL, Feng LQ, Doi Y, Xia Y, Stoesser N, Tian GB. 2017. High rates of human fecal carriage of *mcr-1*-positive multidrug resistant *Enterobacteriaceae* isolates emerge in China in association with successful plasmid families. Clin Infect Dis 66:676–685. https://doi.org/10.1093/cid/cix885.
3. Macesic N, Green D, Wang Z, Sullivan SB, Shim K, Park S, Whittier S, Furuya EY, Gomez-Simmonds A, Uhlemann AC. 2017. Detection of *mcr-1*-carrying *Escherichia coli* causing bloodstream infection in a New York City hospital: avian origins, human concerns? Open Forum Infect Dis 4:ofx115. https://doi.org/10.1093/ofid/ofx115.
4. Macesic N, Gomez-Simmonds A, Sullivan SB, Giddins MJ, Ferguson SA, Korakavi G, Leeds D, Park S, Shim K, Sowash MG, Hofbauer M, Finkel R, Hu Y, West J, Toussaint NC, Greendyke WG, Miko BA, Pereira MR, Whittier S, Verna EC, Uhlemann AC. 2018. Genomic surveillance reveals diversity of multidrug-resistant organism colonization and infection: a prospective cohort study in liver transplant recipients. Clin Infect Dis 67:905–912. https://doi.org/10.1093/cid/ciy199.
5. CLSI. 2015. Performance standards for antimicrobial susceptibility testing; 25th informational supplement. Document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.
6. Nüesch-Inderbinen MT, Kayser FH, Hächler H. 1997. Survey and molecular genetics of SHV beta-lactamases in *Enterobacteriaceae* in Switzerland: two novel enzymes, SHV-11 and SHV-12. Antimicrob Agents Chemother 41:943–949. https://doi.org/10.1128/AAC.41.5.943.
7. Shenoy ES, Pierce VM, Walters MS, Moulton-Meissner H, Lawsin A, Lonsway D, Shugart A, McAllister G, Halpin AL, Zambrano-Gonzalez A, Ryan EE, Suslak D, DeJesus A, Barton K, Madoff LC, McHale E, DeMaria A, Hooper DC. 2018. Transmission of mobile colistin resistance (*mcr-1*) by duodenoscope. Clin Infect Dis https://doi.org/10.1093/cid/ciy683.
8. Kaiser T, Finstermeier K, Hantzsch M, Faucheux S, Kaase M, Eckmanns T, Bercker S, Kaisers UX, Lippmann N, Rodloff AC, Thiery J, Lubbert C. 2018. Stalking a lethal superbug by whole-genome sequencing and phylogenetics: influence on unraveling a major hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae*. Am J Infect Control 46:54–59. https://doi.org/10.1016/j.ajic.2017.07.022.
9. Chan WS, Au CH, Ho DN, Chan TL, Ma ES, Tang BS. 2018. Prospective study on human fecal carriage of *Enterobacteriaceae* possessing *mcr-1* and *mcr-2* genes in a regional hospital in Hong Kong. BMC Infect Dis 18:81. https://doi.org/10.1186/s12879-018-2987-y.