Chromosomal Locations of *mcr-1* and $bla_{CTX-M-15}$ in Fluoroquinolone-Resistant *Escherichia coli* ST410

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DOI: http://dx.doi.org/10.3201/eid2209.160692

To the Editor: Recently, Yi-Yun Liu et al. reported on the discovery of *mcr-1*, a plasmidborne resistance gene mediating resistance to colistin, in isolates obtained from humans and animals (*1*). Since the original publication, *mcr-1* with or without the insertion element IS*Apl1* has been detected on plasmids of different incompatibility groups, including IncI2, IncHI2, and IncX4, and in many different countries (*1–3*). Because colistin is a last-resort parenteral antimicrobial drug, the transfer of *mcr-1* by

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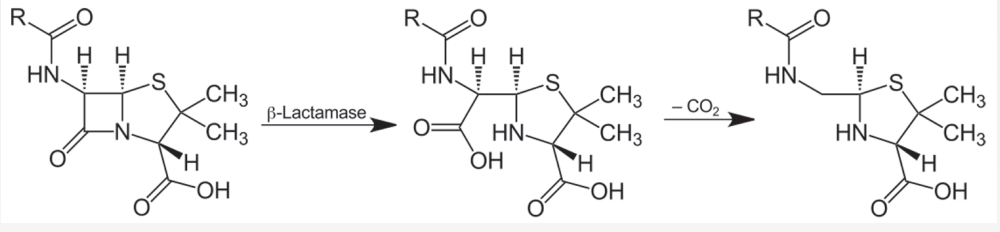
etymologia

β-Lactamase [ba'tə lak'tə-mās]

Enzymes that catalyze the cleavage of β-lactam rings in penicillins, cephalosporins, monobactams, and carbapenems were first described by Abraham and Chain in 1940. These enzymes confer resistance to β-lactam antibiotics on bacteria that produce them. β-lactamases are ancient, theorized to have evolved 1–2 billion years ago, but the emergence and spread of penicillin-resistant staphylococci in hospitals in the 1950s showed how penicillin use could select producers from a population of nonproducers. "Lactam" is a portmanteau of "**lac**tone" (from the Latin *lactis*, "milk," since lactic acid was isolated from soured milk) and "**am**ide." The "β" refers to the nitrogen's position on the second carbon in the ring. The suffix "-ase," indicating an enzyme, is derived from "diastase" (from the Greek *diastasis*, "separation"), the first enzyme discovered in 1833 by Payen and Persoz.



Action of β-lactamase and decarboxylation of the β-lactam ring. Equation by Jü, own work, public domain, https://commons.wikimedia.org/w/index.php?curid=11204303

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DOI: http://dx.doi.org/10.3201/eid2209.ET2209

conjugation or through mobilizable plasmids raises concern about the emergence of pan-resistant *Enterobacteriaceae*.

We previously described extended-spectrum β-lactamase (ESBL)–producing and carbapenemase-producing isolates obtained from livestock and a human in Germany that harbored the *mcr-1* gene (*2*). Because the transfer of *mcr-1* through the food chain is highly likely, we looked for its presence in 62 whole-genome sequenced ESBL-producing *Escherichia coli* isolates obtained during 2012–2013 from food products sampled in Germany (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/8/16-0692-Techapp1.pdf). We detected 4 isolates harboring the *mcr-1* gene (*E. coli* RL138, RL145, RL158, and RL465) that displayed a colistin MIC of 4 mg/L (online Technical Appendix Table 1). The raw sequencing reads and the assembled contigs of the *mcr-1*–positive isolates were deposited in the European Nucleotide Archive under project accession no. PRJEB13470. We conducted conjugation experiments to analyze the transferability of *mcr-1* (online Technical Appendix). For all isolates except RL465, *mcr-1* was transferable to *E. coli* J53 *Az*^{r}. For isolates RL138, RL145, and RL158, the *mcr-1* gene was present on IncX4 and IncHI2 plasmids (Figure, panel A, http://wwwnc.cdc.gov/EID/article/22/8/16-0692-F1.htm; online Technical Appendix Table 2). The sequence type (ST) 410 *E. coli* isolate RL465 was detected in a turkey hen meat sample from 2013 and harbored $bla_{\text{CTX-M-15}}$ and *mcr-1*, a gene combination hitherto identified only in travelers from the Netherlands and children from China (*4*). Both the $bla_{\text{CTX-M-15}}$ and *mcr-1* genes were not transferable, indicating that neither gene was plasmid-encoded. Examination of the genetic environment of *mcr-1* in the assembled gapped genome showed a chromosomal location for the *mcr-1* transposition unit that included an IS*Apl1* element (Figure 1, panel A; online Technical Appendix Figure 1, panel A) flanked by the inverted repeats (IR-R1, IR-R2, and IR-L1). We verified the chromosomal location for the *mcr-1* gene by sequencing the genome to completion, using long-read single-molecule real-time sequencing (Pacific Biosciences, Menlo Park, CA, USA; online Technical Appendix Figure 2); the resulting contigs of *E. coli* RL465 were deposited in the European Nucleotide Archive under accession no. PRJEB14095. One copy of the IS*Apl1-mcr-1* transposition unit was located in the region between a predicted 4Fe-4S ferredoxin-type protein (*ydhY*) and *ldtE* (L,D-transpeptidase) (bp 2652307–2665241), and flanked on either side by a 2-bp direct repeat (CA). We observed a similar situation for the IS*Ecp1*-$bla_{\text{CTX-M-15}}$–*orf477* transposition unit (online Technical Appendix Figure 1, panel B). However, this insertion mapped to a different chromosomal location in a region encoding a defective lambdoid prophage inserted between the molybdate ABC transporter operon (*modABC*) and the biotin biosynthesis operon (*bioABCDF*) (bp 1662140–1716472). It was flanked by direct repeats (TGGTT).

We reexamined our collection of 424 genome sequenced ESBL- and carbapenemase-encoding *E. coli* isolates, obtained during 2010–2014 (*2*), for isolates that harbored $bla_{\text{CTX-M-15}}$ at a chromosomal location identical to that found in *E. coli* RL465. We detected 3 such isolates from 2010–2011 from companion animals and livestock (R107, sock swab dairy cattle farm, 2011; R208, sock swab pig fattening farm, 2011; V177, sick dog, 2010), and 11 consecutive isolates from a hemato-oncologic patient (*5*), obtained within an 11-month period during 2011–2012 (E006910, E007337, E007651, E007825, E000565, E002592, E002816, E003488, E005417, E006587, E006874) (Figure, panel B). All of these isolates were ST410 and negative for the *mcr-1* gene. Phylogenetic analysis of the core genome of these isolates with *E. coli* RL465 using the program Harvest Suite (*6*) indicated they were highly related and separated from *E. coli* V177 (the oldest isolate) by 66 (E006910, E007651) to 110 (E007337) single-nucleotide polymorphisms (core genome size 94%, representing 4.58 Mbp). Thus, our results suggest that transposition of the IS*Apl*1-*mcr-1* unit to the chromosome in *E. coli* RL465 is a later event and probably occurred after transfer of the $bla_{\text{CTX-M-15}}$ allele to the distinct chromosomal location into this *E. coli* ST410 subclone.

These findings highlight 2 independent points. First, our results extend data on the mobility of IS*Apl*1-*mcr-1* to a chromosomal location and reveal a new dimension in the transmissible nature of *mcr-1* in colistin-resistant *Enterobacteriaceae* isolates and their ecology. Second, clonal isolates of ST410 have been isolated from diverse environments, livestock, companion animals, and humans and, as we demonstrate here, in turkey hen meat (*7*,*8*). Thus, the simultaneous spread of the *mcr-1* and $bla_{\text{CTX-M-15}}$ genes mediated by a single bacterial clone is real and suggests that *mcr-1* is already present in the diverse reservoirs inhabited by these isolates.

Acknowledgments

We thank Christina Gerstmann, Natalia Lest, Simone Severitt, and Nicole Mrotzek for excellent technical assistance and Geovanna Brenner Michael, Yvonne Pfeifer, Axel Hamprecht, Lena Biehl, and Martin Kaase for providing the nonfood isolates.

This study was supported by grants to the German Center of Infection Research (DZIF), and the Zoonoses Network "ESBL and fluoroquinolone resistance in Enterobacteriaceae (RESET)" Consortium through the German Federal Ministry of Education and Research (BMBF; grant numbers 8000 701–3 [HZI], 01KI1013G and 01KI1313G to T.C. and C.I., and TI06.001 and 8032808811 to T.C.).

L.F., C.I., and T.C. conceived the study; S.E.W., K.G., H.G., S.D., B.B., C.S., and J.O. performed experiments; A.I., J.F., H.S., B.G., and A.K. contributed isolates and reagents; L.F., B.B., C.I., and T.C. analyzed the data; and T.C. and L.F. wrote the manuscript, which all authors approved.

B.G. is currently employed with the European Food Safety Authority (EFSA) in its BIOCONTAM Unit that provides scientific and administrative support to EFSA's scientific activities in the area of Microbial Risk Assessment. The positions and opinions presented in this article are those of the authors alone and are not intended to represent the views or scientific works of EFSA. The other authors have nothing to proclaim.

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Specificity of Dengue NS1 Antigen in Differential Diagnosis of Dengue and Zika Virus Infection

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DOI: http://dx.doi.org/10.3201/eid2209.160725

To the Editor: Circulation of new arboviruses of the genus *Flavivirus* poses a major problem for differential diagnosis. Zika virus, a mosquitoborne virus of the family *Flaviviridae*, is closely related to other arboviruses circulating in the Americas, including dengue, yellow fever, Saint Louis encephalitis, and West Nile viruses (*1*,*2*). Serologic cross-reactivity between these arboviruses is common; thus, to ensure optimal patient care and accurate epidemiologic surveillance, an effective differential diagnosis is required in regions with active transmission of dengue virus and circulation of Zika virus (*2–4*).

Cross-reactivity between flaviviruses has been reported in antibody assays and in tests for Dengue nonstructural 1 glycoprotein (NS1) antigen. Gyurech et al. (*5*) reported false-positive test results for dengue NS1 antigen in a patient with acute Zika virus infection. Of the 3 NS1 tests used in that study, only the SD Bioline Dengue Duo (Standard Diagnostics, Inc., Gyeonggi-do, South Korea) showed positive results for 3 of 4 sequential serum samples from the patient.

Cross-reactivity in NS1 dengue tests (ELISA and immunochromatographic) using serum samples from patients with acute Zika virus infection would have medically significant consequences. We therefore conducted a retrospective analysis of the differential diagnosis for dengue and Zika virus infections since the beginning of the Zika virus outbreak in French Guiana, a department of France on the northeast coast of South America.

French Guiana is subject to endemoepidemic circulation of dengue and experienced a large outbreak of chikungunya in 2014. We conducted our study from December 17, 2015 (the time of biologic confirmation of the first case of Zika virus disease in French Guiana), through March 2, 2016. During that time, the incidence of dengue virus infection in French Guiana was low, and only 1 sporadic case was confirmed. We studied clinical samples collected during this period from all patients with suspected arbovirus infection.