Chromosomal Locations of mcr-1 and blaCTX-M-15 in Fluoroquinolone-Resistant Escherichia coli ST410

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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 ISApl1 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...
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://www.ncbi.nlm.nih.gov/pubmed/23943497). 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 Az2+. For isolates RL138, RL145, and RL158, the mcr-1 gene was present on IncX4 and IncHI2 plasmids (Figure, panel A, http://www.ncbi.nlm.nih.gov/pubmed/23943497; 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_{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_{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 ISAp1 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 ISAp1-mcr-1 transposition unit was located in the region between a predicted 4Fe-4S ferredoxin-type protein (ydhY) and ldE (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 ISEcp1-bla_{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 (bioBCDF) (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_{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 ISAp1-mcr-1 unit to the chromosome in E. coli RL465 is a later event and probably occurred after transfer of the bla_{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 ISAp1-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_{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.

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B.G. is currently employed with the European Food Safety Authority (EFSA) in its BIOCOSTAM 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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To the Editor: Circulation of new arboviruses of the genus Flavivirus poses a major problem for differential diagnosis. Zika virus, a mosquito-borne 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-negative 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.