

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

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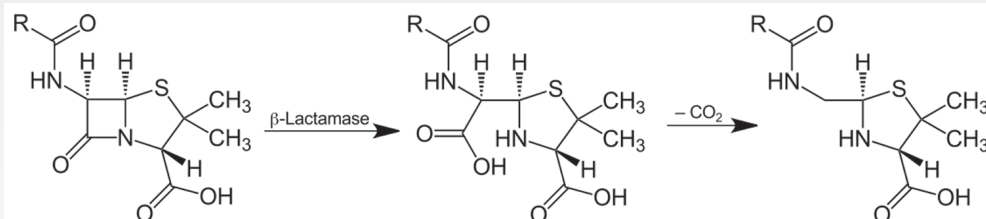
To the Editor: Recently, Yi-Yun Liu et al. reported on the discovery of *mcr-1*, a plasmidborne resistance gene mediating resistance to colistin, in isolates obtained from humans and animals (1). Since the original publication, *mcr-1* with or without the insertion element IS*AplI* has been detected 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 “lactone” (from the Latin *lactis*, “milk,” since lactic acid was isolated from soured milk) and “amide.” 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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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*_{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 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 (*vdhY*) 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*_{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 lambdaoid 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*_{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*Apl1*-*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 IS*Apl1*-*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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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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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-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.

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

Technical Appendix

Collection of Isolates

Retail food samples originating from cattle, swine, and poultry (meat and milk) were sampled by food inspectors in 4 different regions of Germany during May 2012–April 2013. All samples were unrelated to each other. No information was recorded about the country from which the animals originated. Extended-spectrum β -lactamases (ESBL)–producing isolates were selected by using MacConkey agar plates supplemented with 1 mg/L cefotaxime. A subset of 62 *Escherichia coli* isolates was analyzed by using whole-genome sequencing.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed by using VITEK 2 (AST-card: N248; bioMérieux, Nürtingen, Germany). Colistin susceptibility testing was performed by using broth microdilution according to European Union Commission Implementing Decision 2013/652/EU (<http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32013D0652>) (using EUVSEC Sensititer plates, Trek Diagnostic systems, Thermo Fischer Scientific, Dreieich, Germany). The results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (2015) and Clinical and Laboratory Standards Institute (2015) guidelines (1,2).

Whole-Genome Sequencing

Whole-genome DNA was isolated from overnight cultures by using the Purelink Genome DNA Mini kit (Invitrogen, Darmstadt, Germany). The sequencing library was produced by using Illumina Nextera XT Kit and sequenced on a MiSeq instrument (Illumina, San Diego, CA,

USA), with 2×300 read length. The average read length accounted for 180 nt with an average coverage of $51\times$. Raw reads were assembled by using SPAdes (v. 3.0) (3). To confirm the chromosomal location of *mcr-1* in *E. coli* RL465, long-read single-molecule real-time (SMRT) sequencing was performed. For this, DNA was isolated by using the method described by Pitcher et al. (4). A SMRTbell template library was prepared according to the instructions, following the Procedure & Checklist—10 kb Template Preparation Using BluePippin Size-Selection System. Briefly, for preparation of 15-kb libraries 8 μ g genomic DNA was sheared by using g-tubes (Covaris, Woburn, MA, USA) according to the manufacturer's instructions. DNA was end-repaired and ligated overnight to hairpin adapters applying components from the DNA/Polymerase Binding Kit P6 (Pacific Biosciences, Menlo Park, CA, USA). Reactions were conducted according to the manufacturer's instructions. BluePippin Size-Selection to 4 kb was performed according to the manufacturer's instructions (Sage Science, Beverly, MA, USA). Conditions for annealing of sequencing primers and binding of polymerase to purified SMRTbell template were assessed with the Calculator in RS Remote (Pacific Biosciences). SMRT sequencing was conducted on the PacBio RSII (Pacific Biosciences) taking one 240-min movie for a single SMRT cell. We assembled PacBio reads using RS_HGAP_Assembly.3 protocol included in the SMRT Portal 2.3.0. The number of reads from PacBio sequencing accounted for 93,593 with a mean read length of 12,057 nt. To obtain a high-quality genome sequence, we mapped paired-end reads from Illumina sequencing using Burrows–Wheeler Aligner (5). The chromosome displayed a size of 4,894,900 bp ($167\times$ coverage). One plasmid of 157,187 bp ($57\times$ coverage), and 2 phage-like elements 89,746 bp, (Element 1, $39\times$ coverage, circular), and 61,544 bp (Element 2, $234\times$ coverage, linear) were detected.

In Silico Analyses

We identified resistance genes using ResFinder (6), virulence genes using VirulenceFinder (7), plasmid incompatibility groups and plasmid multilocus sequence typing with PlasmidFinder and pMLST (8), and multilocus sequence types using MLST 1.8, according to the scheme of Wirth et al. (9,10). The genetic environment of *mcr-1* and *bla*_{CTX-M-15} was identified using blastn and ISFinder (11,12). Annotation of the *E. coli* RL465 genome and extrachromosomal units was performed using RAST (13). To identify phages, we used the program PHAST (14).

Conjugation Experiments

We conducted conjugation experiments at 37°C or at ambient temperatures as described previously (15) using *E. coli* J53 Az^r as a recipient and 2 mg/L colistin sulfate and 200 mg/L sodium azide as selective agents. Replicon typing of the transconjugants was performed as described in the literature (16,17).

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Technical Appendix Table 1. Depiction of the MIC of the *mcr-1*-encoding and extended-spectrum β -lactamase ESBL-producing isolates from retail food*

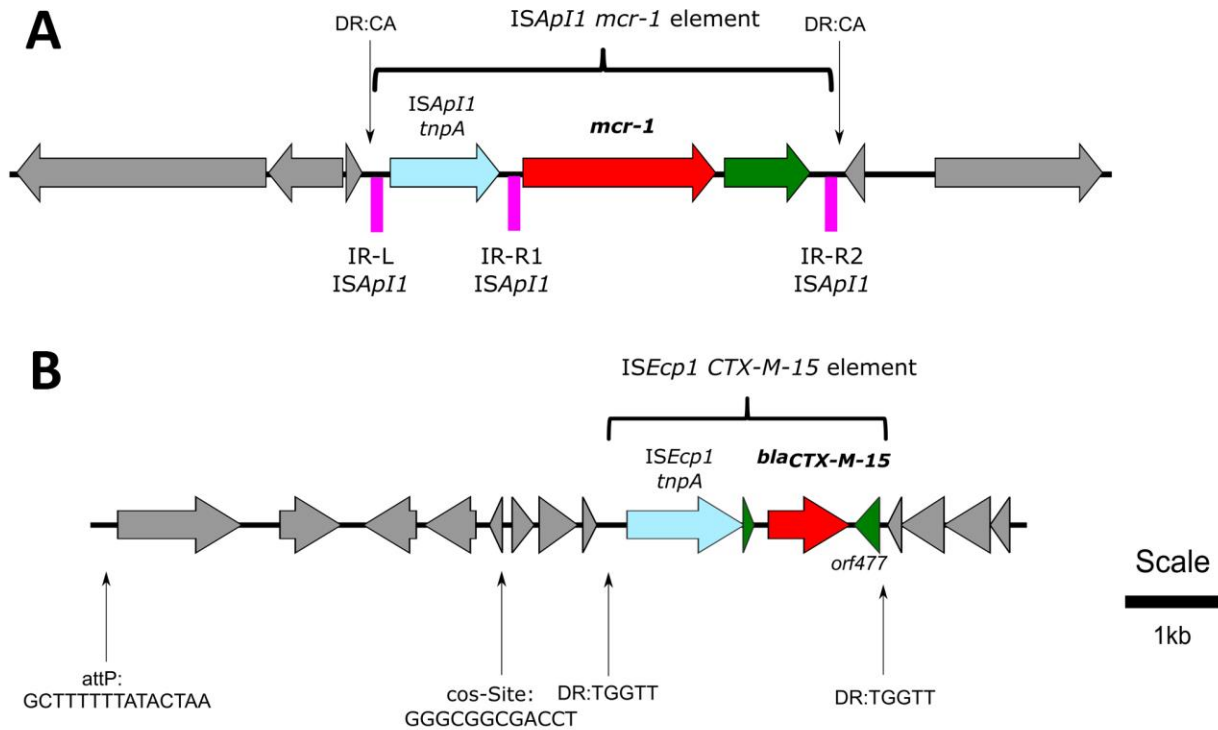
Antimicrobial drug class, drug	RL138		RL145		RL158		RL465	
	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation
Aminoglycosides								
Amikacin	≤2	S	≤2	S	≤2	S	≤2	S
Gentamicin	≤1	S	≤1	S	≤1	S	≤1	S
Tobramycin	≤1	S	≤1	S	≤1	S	≤1	S
Penicillins								
Ampicillin	≥32	R	≥32	R	≥32	R	≥32	R
Ampicillin/Sulbactam	16	R	≥32	R	16	R	16	R
Piperacillin	≥128	R	≥128	R	≥128	R	≥128	R
Piperacillin/Tazobactam	≤4	S	8	S	≤4	S	≤4	S
Carbapenems								
Ertapenem	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S
Imipenem	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S
Meropenem	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S
Cephalosporins								
Cefepime	2	I	≤1	S	2	I	2	I
Cefotaxime	8	R	8	R	8	R	32	R
Cefpodoxime	≥8	R	≥8	R	≥8	R	≥8	R
Ceftazidime	≤1	S	16	R	≤1	S	4	R
Cefuroxime	≥64	R	≥64	R	≥64	R	≥64	R
Fluoroquinolones								
Ciprofloxacin	≥4	R	≤0.25	S	≤0.25	S	≥4	R
Moxifloxacin	≥8	R	≤0.25	S	≤0.25	S	≥8	R
Miscellaneous agents								
Fosfomycin	≤16	S	≤16	S	≤16	S	≤16	S
Trimethoprim/sulfamethoxazole	≥320	R	≤20	S	≥320	R	≥320	R
Monobactams: Aztreonam								
	≤1	S	2	I	2	I	16	R
Tetracyclines								
Tetracycline	≥16	R	≥16	R	≤1	R	≥16	R
Tigecycline	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S

*The MIC results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (2015) and the Clinical and Laboratory Standards Institute (2015) guidelines (1,2). MIC, minimal inhibitory concentration; R, resistant; S, susceptible.

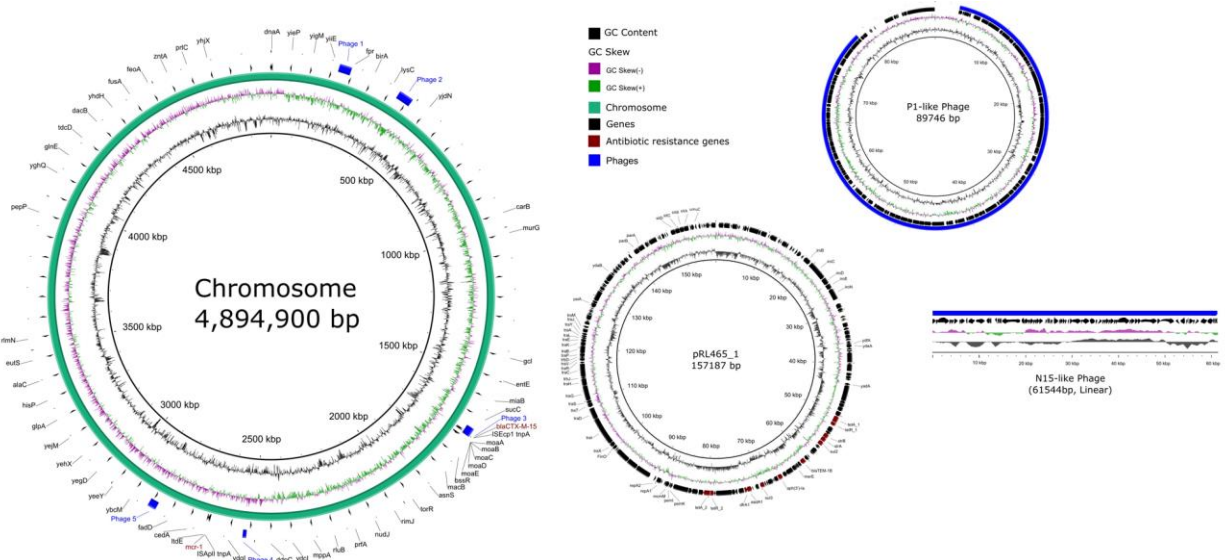
Technical Appendix Table 2. Characteristics of the *mcr-1*-encoding ESBL-producing *Escherichia coli* isolates from retail meat*

Variable	Characteristic			
	RL138	RL145	RL158	RL465
Name	RL138	RL145	RL158	RL465
Species	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
Sequence type	ST602	ST88	ST357	ST410
Year of isolation	2012	2012	2012	2013
Colistin MIC	4 mg/L	4 mg/L	4 mg/L	4 mg/L
Source	Chicken breast	Minced meat, beef	Turkey hen Schnitzel	Turkey hen meat
Antimicrobial resistance genes	<i>aadA1</i> , <i>aadA2</i> -like, <i>bla</i> _{CTX-M-14} , <i>catA1</i> -like, <i>cmiA1</i> -like, <i>dfrA1</i> , <i>mcr-1</i> , <i>strA</i> -like, <i>strB</i> , <i>sul1</i> , <i>sul3</i> , <i>tet(A)</i>	<i>aph(3')-Ia</i> -like, <i>bla</i> _{OXA-1} , <i>bla</i> _{TEM-52C} , <i>mcr-1</i> , <i>sul1</i> , <i>tet(B)</i>	<i>aadA1</i> , <i>bla</i> _{CTX-M-1} , <i>dfrA1</i> , <i>mcr-1</i> , <i>sul1</i> -like, <i>sul2</i>	<i>aadA1</i> , <i>aph(3')-Ia</i> , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1B} , <i>dfrA1</i> , <i>mcr-1</i> , <i>strA</i> -like, <i>strB</i> -like, <i>sul2</i> , <i>sul3</i> , <i>tet(A)</i> -like
Virulence genes	<i>iroN</i> , <i>iss</i> , <i>lpfA</i> , <i>mchF</i> , <i>tsh</i>	<i>iha</i> , <i>iroN</i> , <i>iss</i> , <i>lpfA</i> , <i>mchF</i> , <i>mcmA</i>	<i>iroN</i> , <i>iss</i> , <i>mchF</i>	<i>cma</i> , <i>iroN</i> , <i>iss</i> , <i>lpfA</i>
Plasmid incompatibility groups	IncHI2, IncFIC(FII), IncHI2A, IncI1, IncFIB(AP001918), IncFIA, IncP, Col(MG828)	IncFIB(AP001918), IncI1, IncFIC(FII), IncI2, IncX4, ColRNAI	IncFIC(FII), IncFIA, IncI1, IncFIB(AP001918), ColRNAI, IncX4, Col(MG828)	IncFIB(AP001918), IncFII(pCoo), Col156, ColRNAI
pMLST	IncHI2[ST-4], IncI1(unknown sequence type), IncF[F18:A6*:B1]	IncI1[ST-36], IncF[F18:A-B1]	IncF[F18:A6*:B1], IncI1[ST-7]	IncF [F16:A-B1]
<i>mcr-1</i> location	IncHI2 plasmid	IncX4 plasmid	IncX4 plasmid	Chromosome

*MIC, minimal inhibitory concentration; pMLST, plasmid multilocus sequence type.



Technical Appendix Figure 1. Genetic environments of the chromosomally located antimicrobial resistance genes (A) *mcr-1* and (B) *bla*_{CTX-M-15} of the *Escherichia coli* isolate RL465. *attR*, right phage attachment site; DR, direct repeat; cos site, cohesive end sequence of prophage; IR, inverted repeats. Genes marked in red display an antimicrobial resistance gene; in light blue transposase genes from the transposition units, green, other genes in the transposition units, pink bars depict the presence of the inverted repeats of ISAp11. Unrelated flanking genes are shaded gray.



Technical Appendix Figure 2. Schematic depiction of the chromosome, its IncFII/FIB plasmid and the 2 phage elements in *Escherichia coli* RL465.