



Full Genome Sequence of pT3, a Multiresistant Plasmid Carrying the *mcr-3.5* Colistin Resistance Gene, Recovered from an Extended-Spectrum- β -Lactamase-Producing *Escherichia coli* Isolate from Crickets Sold as Food

Katrin Zurfluh,^a Marc J. A. Stevens,^a Melissa Bucher,^a Laurent Poirel,^{b,c} Patrice Nordmann,^{b,c}  Roger Stephan^a

^aInstitute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

^bEmerging Antibiotic Resistance, Medical and Molecular Microbiology Unit, Department of Medicine, University of Fribourg, Fribourg, Switzerland

^cNational Reference Center for Emerging Antibiotic Resistance, University of Fribourg, Fribourg, Switzerland

ABSTRACT Here, we sequenced multidrug resistance plasmid pT3, which encodes *mcr-3.5* and other resistance genes. The cooccurrence of *mcr* genes and other resistance genes on a single plasmid is of concern.

The last decade has seen a rapid and massive global spread of extended-spectrum- β -lactamase (ESBL)-producing *Enterobacteriaceae* in many environments, including food (1).

Recently, we isolated an ESBL-producing *Escherichia coli* strain from crickets of the family Gryllidae that were bought as food in a Thai store in Switzerland. The food sample was homogenized using a stomacher sample blender and incubated at a 1:10 ratio in *Enterobacteriaceae* enrichment (EE) broth (BD, Franklin Lakes, NJ, USA) at 37°C overnight. For the detection of ESBL producers, chromogenic Brilliance ESBL agar (Oxoid, Hampshire, United Kingdom) was inoculated with one loopful of the enrichment culture. The plate was incubated at 37°C for 24 h under aerobic conditions.

One presumptive-positive *E. coli* colony on the chromogenic agar was identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Biotyper compass explorer software 4.1.60; Bruker Daltonics, Bremen, Germany) and further characterized according to Zurfluh et al. (2).

The strain was characterized as a CTX-M-55-producing *Escherichia coli* strain belonging to phylogroup A and showing phenotypical resistance to colistin (MIC, 8 mg/liter), ampicillin, cefazolin, cefotaxime, sulfamethoxazole-trimethoprim, kanamycin, gentamicin, tetracycline, and chloramphenicol.

From this strain, plasmids were extracted from a 1-ml overnight culture in brain heart infusion (BHI) broth (Oxoid, Cheshire, England) using the Qiagen midikit (Qiagen, Hombrechtikon, Switzerland) and transferred by transformation using electroporation (3) into *E. coli* DH5 α (Molecular Cloning Laboratories [MCLAB], South San Francisco, CA, USA). Colistin-resistant transformants were selected on LB agar supplemented with 2 mg/liter colistin (Sigma-Aldrich, Buchs SG, Switzerland). From a colistin-resistant transformant, the plasmid pT3 was extracted (250 ng/ μ l) using a large-construct kit (Qiagen) according to the manufacturer's protocol. Libraries were prepared using the SMRTbell template prep kit 1.0. according to the manufacturer's instruction. Sequencing was done using a single PacBio RS II cell (Pacific Biosciences, Menlo Park, CA, USA) and performed at the Functional Genomics Center Zurich (Zurich, Switzerland). The sequencing resulted in 150,292 reads with a total length of 1,738,030,792 bp. Raw reads were assembled using Hierarchical Genome Assembly Process (HGAP) 3.0 in SMRT Portal 3.2 (Pacific Biosciences) using 0.88 as the polymerase read quality cutoff, 100 kb

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Address correspondence to Roger Stephan, stephanr@fsafety.uzh.ch.

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as the genome size, and all other options left unchanged. The polymerase read quality filtering resulted in 1,873 input reads with a total length of 30,356,970 bp. A total of 1,843 of the input reads could be assembled to a single primary contig of 107,601 bp. The primary contig was polished by deleting the repetitive sequence at the contig end, which resulted in a 79,291-bp circular sequence with an approximate coverage of 270-fold and a GC content of 49.7%.

Comparison of pT3 with the plasmid database PlasmidFinder (4) revealed that pT3 belongs to the IncF1A, IncF1B, and IncX1 plasmid incompatibility groups and lacked the genes needed for conjugation. The plasmid was annotated with the NCBI Prokaryotic Genome Annotation Pipeline. It harbors the colistin resistance gene *mcr-3.5*, which is the first time that this gene has been found in Switzerland. Furthermore, pT3 harbors *tet(A)*, *bla*_{TEM-128}, and *qnrS1* genes, encoding resistance to tetracycline, β -lactams, and quinolones, respectively. In addition, genes encoding resistances to silver, copper, and mercury were located on pT3. Comparison to plasmids in public databases revealed that pT3 is a mosaic plasmid. Mosaic plasmids are composed of genetic elements from distinct sources and are common in *Enterobacteriaceae* members (5).

A high similarity between pT3 and plasmids from different species was noteworthy, and the insertion of the *mcr-3.5* gene onto such plasmids presents a potential additional threat. The cooccurrence of *mcr* genes and other resistance genes on a single plasmid is of concern.

Data availability. The full sequence of pT3 has been deposited in the GenBank database under the accession number [MK656937](#). Reads are available in the Sequence Read Archive (SRA) under study number [PRJNA527974](#), experiment number [SRX5905084](#), and run number [SRR9131260](#).

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REFERENCES

- Müller A, Stephan R, Nüesch-Inderbilen M. 2016. Distribution of virulence factors in ESBL-producing *Escherichia coli* isolated from the environment, livestock, food and humans. *Sci Total Environ* 541:667–672. <https://doi.org/10.1016/j.scitotenv.2015.09.135>.
- Zurfluh K, Nüesch-Inderbilen M, Morach M, Zihler Berner A, Hächler H, Stephan R. 2015. Extended-spectrum- β -lactamase-producing *Enterobacteriaceae* in vegetables imported from the Dominican Republic, India, Thailand and Vietnam. *Appl Environ Microbiol* 81:3115–3120. <https://doi.org/10.1128/AEM.00258-15>.
- Shigekawa K, Dower WJ. 1988. Electroporation of eukaryotes and prokaryotes: a general approach to the introduction of macromolecules into cells. *Biotechniques* 6:742–751.
- Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Aarestrup FM, Hasman H. 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 58:3895–3903. <https://doi.org/10.1128/AAC.02412-14>.
- Pesesky MW, Tilley R, Beck D. 2019. Mosaic plasmids are abundant and unevenly distributed across prokaryotic taxa. *Plasmid* 102:10–18. <https://doi.org/10.1016/j.plasmid.2019.02.003>.