



Letter to the Editor

First report of an *mcr-1*-harboring *Salmonella enterica* subsp. *enterica* serotype 4,5,12:i:- strain isolated from blood of a patient in Switzerland


Sir,

In the past, colistin use has been mostly limited to veterinary medicine owing to its rather severe side effects, but given the increase in multidrug-resistant Gram-negative bacterial infections, the World Health Organization (WHO) recently re-labelled colistin as a 'critically important antibiotic'. The first description of the plasmid-borne mobilizable colistin resistance gene *mcr-1* in 2015 [1] caused great concern, as the ease of potential spread on a conjugative plasmid encoding resistance to polymyxins might change the resistance situation to colistin drastically. In line with this, *mcr-1*-mediated colistin resistance in Enterobacteriaceae, including *Salmonella enterica*, has since been reported from a wide range of geographical locations [2]. Here we report the first case of *mcr-1*-harboring *S. enterica* in Switzerland.

Salmonella enterica subsp. *enterica* serotype 4,5,12:i:- strain N17-0346 was isolated in 2017 from the blood of a 77-year-old male patient in Switzerland with no known travel history. The serotype was determined by the Swiss National Reference Centre for Enteropathogenic Bacteria and Listeria (NENT) according to the White–Kauffmann–Le Minor scheme. The strain was subjected to antimicrobial susceptibility testing to 16 antimicrobial agents by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) protocols and the results were evaluated according to CLSI criteria [3]. Determination of the colistin minimum inhibitory concentration (MIC) was performed by the broth microdilution method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (<http://www.eucast.org>). Strain N17-0346 was phenotypically resistant to colistin (MIC = 4 mg/L), ampicillin and streptomycin but was susceptible to amoxicillin/clavulanic acid, cefazolin, cefotaxime, cefepime, gentamicin, kanamycin, nalidixic acid, ciprofloxacin, trimethoprim/sulfamethoxazole, azithromycin, fosfomycin, nitrofurantoin, tetracycline and chloramphenicol.

Strain N17-0346 was sequenced using a MiSeq platform (Illumina Inc., San Diego, CA) and a Nextera XT Library Kit (Illumina Inc.) utilising either 500 or 600 cycles of paired-end reads. De novo assembly using CLC Genomics Workbench v.9.0 (CLC Bio, Aarhus, Denmark) resulted in a genome size of 4 981 418 bp with 95 contigs and a GC content of 52.1%. The genome was annotated using the Rapid Annotation using Subsystem Technology (RAST) annotation server and 5061 coding sequences (CDS) were identified. In silico seven-gene multilocus sequence typing (MLST) using seq2mlst v.1.0.1 (<https://github.com/lmc297/seq2mlst>) identi-

fied strain N17-0346 as ST34. Core-genome MLST using the command-line implementation of SISTR v.1.0.2 classified it as ST3833327333. Genome-wide detection of antimicrobial resistance (AMR) genes using the method described and validated for *Salmonella* Typhimurium by Carroll et al. [4] and implemented in BTypper v.2.2.0 (<https://github.com/lmc297/BTypper>) in conjunction with the ARG-ANNOT AMR gene database, PlasmidFinder and PlasFlow v.1.0 produced the following hits: *mcr-1* encoded on an IncX4 plasmid (pN17-0346) with no further AMR genes on the same contig; *strA/strB* (conferring streptomycin resistance) and *sullII* (encoding sulfonamide resistance) located on an IncQ1 plasmid; *bla*_{TEM-30} (coding for an inhibitor-resistant β -lactamase) on a contig classified as belonging to a plasmid; and *aac(6')-Iaa* (aminoglycoside acetyltransferase) encoded on the chromosome.

To generate a closed plasmid sequence of the *mcr-1*-coding sequence pN17-0346, the plasmid was sequenced using a Nextera DNA Flex Sample Preparation Kit on an Illumina MiniSeq Sequencer with 150-bp paired-end reads, yielding the closed 34 092-bp plasmid sequence with a per base coverage of $>50\times$ for all bases. RAST annotation determined 51 CDS and a partial insertion sequence (IS) element. The *mcr-1*-coding sequence was located directly upstream of an open reading frame encoding a hypothetical protein with similarities to a PAP2 superfamily protein that is frequently seen in association with *mcr-1* [5]. A single copy of an incomplete version of IS*Apl1* was located downstream of the *mcr-1* cassette, but no IS*Apl1* element was identified upstream of it.

Conjugal transfer of the *mcr-1*-harboring IncX4 plasmid from donor strain N17-0346 to the recipient *Escherichia coli* HK225 (streptomycin- and rifampicin-resistant) was tested at 25°C and 37°C in liquid medium as well as on solid agar plates. Transconjugants carrying the *mcr-1* gene as confirmed by PCR were found in all of these experiments, with a conjugation efficiency of 2.2×10^{-4} per donor cell on solid agar at 37°C. The parent *E. coli* HK225 had a colistin MIC of 1 mg/L; after conjugation of the IncX4 plasmid, the MIC increased to 4 mg/L. To our knowledge, this is the first report of a *Salmonella* strain carrying an *mcr-1* colistin resistance gene in Switzerland.

The full sequence of strain N17-0346 has been deposited in GenBank under the accession no. **QEAL0000000** and the sequence of the *mcr-1*-harboring plasmid pN17-0346 under accession no. **NZ_CP031291.1**

Acknowledgments

The authors thank Denise Althaus for serotyping strain N17-0346 as well as Ben D. Tall for editing the manuscript.

Funding

This material is based on work supported by the National Science Foundation Graduate Research Fellowship Program [grant no. DGE-1144153 to LMC]. HJ is a post-doctoral research fellow funded by the Oak Ridge Institute for Science and Education (Oak Ridge, TN). In addition, this work was partially funded by the Swiss National Science Foundation [project FNS-407240_177381].

Competing interests

None declared.

Ethical approval

Not required.

References

- [1] Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. 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* 2016;16:161–8.
- [2] Sun J, Zhang H, Liu YH, Feng Y. Towards understanding MCR-like colistin resistance. *Trends Microbiol* 2018;26:794–808. doi:10.1016/j.tim.2018.02.006.
- [3] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 27th ed. Wayne, PA: CLSI; 2017. CLSI supplement M100.
- [4] Carroll LM, Wiedmann M, den Bakker H, Siler J, Warchocki S, Kent D, et al. Whole-genome sequencing of drug-resistant *Salmonella enterica* isolates from dairy cattle and humans in New York and Washington States reveals source and geographic associations. *Appl Environ Microbiol* 2017;83:e00117–40.
- [5] Zurfluh K, Klumpp J, Nüesch-Inderbinen M, Stephan R. Full-length nucleotide sequences of *mcr-1*-harboring plasmids isolated from extended-spectrum- β -lactamase-producing *Escherichia coli* isolates of different origins. *Antimicrob Agents Chemother* 2016;60:5589–91.

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Received 14 May 2018
Accepted 4 August 2018