Complete Genome Sequence of Salmonella enterica subsp. enterica Serovar Paratyphi B Sequence Type 28 Harboring mcr-1

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ABSTRACT In 2015, plasmid-mediated colistin resistance was reported to be caused by a mobilized phosphoethanolamine transferase gene (mcr-1) in Enterobacteriaceae. Here, we announce the complete genome sequence of the earliest d-tartrate-fermenting Salmonella enterica subsp. enterica serovar Paratyphi B isolate harboring mcr-1 from the collection of the German National Reference Laboratory for Salmonella.

A common cause of gastroenteritis worldwide is d-tartrate-fermenting (dTa+) Salmonella enterica subsp. enterica serovar Paratyphi B (formerly known as S. enterica serovar Java) (1). Since the late 1990s, a distinct multidrug-resistant genetic lineage of this zoonotic serovar has been commonly isolated from poultry and poultry products, especially in Germany, the Netherlands, and Belgium (2). Multidrug resistance to up to six classes of antimicrobials of the lineage is caused by a chromosomally located class 2 integron carrying the dfrA1-sat2-aadA1 (Tn7) array of gene cassettes (confering resistance to trimethoprim, streptothricin, and aminoglycosides), a mutation in the quinolone resistance-determining region (QRDR) of the gyrA gene (conferring resistance to quinolones), and occasional acquisition of additional resistance plasmids, including extended-spectrum β-lactamase (ESBL) genes (3, 4). In 2015, the first mobilized colistin resistance gene (mcr-1) was identified in Escherichia coli isolates from China (5) and later reported in a number of other countries worldwide (6). These findings raised a significant public health concern because colistin belongs to the last-resort antibiotics used to treat multidrug-resistant infections.

PCR screening of isolates from the collection of the German National Reference Laboratory for Salmonella revealed that S. Paratyphi B (dTa+) acquired the mcr-1 gene in the year 2008. Our earliest observed mcr-1-positive isolate (08-00436) originated from chicken skin.

A single colony of isolate 08-00436 was picked and cultured for 16 h at 37°C in lysogeny broth supplemented with 2 mg/liter colistin-sulfate (Sigma-Aldrich, Darmstadt, Germany). Genomic DNA was extracted using the PureLink genomic DNA minikit (Invitrogen, Carlsbad, CA, USA). Genome sequencing was performed by the GATC Biotech AG (Constance, Germany) using the PacBio RSII system. A de novo genome assembly based on 55,146 reads from a single-molecule real-time (SMRT) cell (N50, read length, 21,347 bp; mean read length, 14,478 bp; mean read score, 0.86) was performed using the SMRT Analysis (version 2.3.0) software (Pacific Biosciences, USA), resulting in 3 contigs with an average coverage of 143.12× per consensus base. Short-read sequencing using Illumina MiSeq technology was carried out on the same DNA sample. Paired reads were mapped against PacBio contigs using CLC Genomics Workbench 9.5.2. The contigs were closed to circular molecules, and errors in homopolymeric regions were corrected. The resulting genome contains a chromosome (4,751,926 bp)
and two plasmids, pSE08-00436-1 (264,914 bp) and pSE08-00436-2, (54,067 bp) comprising an average G+C content of 52%.

Genome analysis using tools of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org) revealed that the isolate belonged to the multilocus sequence type (MLST) 28 (ST28) and carries two copies of the class 2 integron, as well as additional resistance genes in the chromosome. Further resistance genes, including mcr-1, were located on the multireplicon IncHI2/IncHI2A/IncQ1 plasmid pSE08-00436-1. Initial genome annotation was performed using the automated Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). Analysis of the results revealed 5,071 coding sequences (CDSs) (4,898 coding genes and 173 pseudogenes), as well as 117 RNA genes (22 rRNAs, 83 tRNAs, and 12 noncoding RNAs). Further assessment of the genes involved in resistance, plasmid transfer, and virulence is in progress.

**Accession number(s).** Sequences were deposited in GenBank under the accession numbers CP020492 (chromosome), CP020493 (pSE08-00436-1), and CP020494 (pSE08-00436-2).

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The conclusions, findings, and opinions expressed in this article reflect only the view of the authors and not the official position of the European Food Safety Authority.

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