# Complete Genome Sequences of Three Clinical *Listeria monocytogenes* Sequence Type 8 Strains from Recent German Listeriosis Outbreaks

Martin A. Fischer,[a] Andrea Thürmer,[b] Antje Flieger,[a] Sven Halbedel[a]

aFG11 Division of Enteropathogenic Bacteria and *Legionella*, German Consultant Laboratory for *L. monocytogenes*, Robert Koch Institute, Wernigerode, Germany
bMF2 Genome Sequencing, Robert Koch Institute, Berlin, Germany

**ABSTRACT** We report here the closed genome sequences of three clinical *Listeria monocytogenes* strains of multilocus sequence typing (MLST) sequence type 8 (ST8). These strains are representatives of three separate listeriosis outbreak clusters (Alpha1, Pi4, and Sigma1) that affected Germany between 2012 and 2020.

The bacterium *Listeria monocytogenes* frequently causes foodborne outbreaks of invasive listeriosis (1). In Germany, diagnosing labs routinely send clinical isolates from human infections to the German Consultant Laboratory for *L. monocytogenes* for subtyping (2). Systematic genome sequencing of the isolates from patients and comparison with the isolates from possible food sources are then used for outbreak detection (2).

Three outbreaks recently detected were caused by PCR serogroup IIa clones belonging to multilocus sequence typing (MLST) sequence type 8 (ST8) (3, 4). Using 1,701-locus core-genome MLST (cgMLST), we further assigned cgMLST complex types 1248 (CT1248) (from the outbreak referred to as Alpha1), CT2521 (Sigma1), and CT5004 (Pi4; Table 1) to these clones (3–5). This report announces the completion of closed genome sequences of one representative strain for each of these outbreaks (Table 1).

*L. monocytogenes* strains were cultivated for 16 h in brain heart infusion (BHI) broth at 37°C, and chromosomal DNA was isolated by phenol-chloroform extraction, if not stated otherwise (6). The DNA quality and concentration were determined using a NanoDrop spectrophotometer (Thermo Fisher, Waltham, MA, USA). The species identity was confirmed by multiplex PCR (7).

For long-read sequencing of strain 12-05460 (Alpha1), a SMRTbell template library was generated (Pacific Biosciences, Menlo Park, CA, USA) using the BluePippin size selection system. Sequencing was performed on a PacBio RS system using one single-molecule real-time (SMRT) cell in 240-min movie run mode and components from the DNA sequencing kit 4.0 v2 (GATC Biotech, Constance, Germany). In total, 150,292 raw reads were obtained. Quality filtering and adapter trimming were carried out using SMRT Portal v2.3.0 software (Pacific Biosciences) with default parameters. Following this, 3,773 high-quality long reads remained ($N_{50}$, 21,338 bp). Strain 12-05460 had also been sequenced previously using $2 \times 300$-bp paired-end Illumina sequencing, generating 1,614,580 reads (3).

Long-read libraries for strains 18-04415 (Sigma1) and 19-05816 (Pi4) were prepared using the SQK-RKB004 kit and sequenced on a MinION instrument using a 1D flow cell (Oxford Nanopore Technologies, Oxford, UK), generating 39,630 and 39,557 raw reads, respectively. The reads were quality filtered using NanoFilt (8) with default parameters. This resulted in 28,540 ($N_{50}$, 7,332 bp) and 27,229 ($N_{50}$, 12,075 bp) long reads, respectively. Strain 18-04415 had been sequenced previously on a MiSeq instrument using single-direction $1 \times 150$-bp chemistry, yielding 3,190,919 reads (4). For short-read sequencing, strain 19-05816 was cultivated for 16 h in BHI broth at 37°C. The isolation of chromosomal DNA and library

**TABLE 1** Key characteristics of the genomes sequenced in this study

| Characteristic | Data for strain: | | |
| --- | --- | --- | --- |
| | 12-05460 | 18-04415 | 19-05816 |
| Outbreak | Alpha1 | Sigma1 | Pi4 |
| Source type | Clinical isolate | Clinical isolate | Clinical isolate |
| Source of isolation | Cerebrospinal fluid | Cerebrospinal fluid | Synovial fluid |
| Yr of isolation | 2012 | 2018 | 2019 |
| NCBI accession no. | CP063381 | CP064843 | CP063240 |
| ENA accession no. | SAMEA104485072 | SAMEA6798783 | SAMEA7376280 |
| Sequencing method | PacBio | MinION | MinION |
| ENA accession no. for raw long-read data | ERX4581156 | ERX4581159 | ERX4581158 |
| ENA accession no. for raw short-read data | ERX2313070 | ERX4056512 | ERX4889691 |
| Genome size (bp) | 2,986,724 | 2,951,919 | 2,951,169 |
| GC content (%) | 37.95 | 37.96 | 37.96 |
| No. of protein coding genes | 2,922 | 2,965 | 2,881 |
| No. of rRNA operons | 6 | 6 | 6 |
| No. of tRNA genes | 67 | 67 | 67 |
| Pathogenicity island | LIPI-1 | LIPI-1 | LIPI-1 |
| Plasmid (GenBank accession no.) | None | pLMN1546 (CP064844) | None |
| PCR serogroup | IIa | IIa | IIa |
| ST[a] | ST8 | ST8 | ST8 |
| CT[b] | CT1248 | CT2521 | CT5004 |
| Outbreak duration | 2012–2016 | 2014–2019 | 2017–2020 |
| Reference(s) | 3, 14 | 4 | Unpublished |

[a] ST, sequence type according to seven-locus MLST (15).
[b] CT, complex type according to 1,701-locus cgMLST (5).

preparation were performed as described previously (4). Sequencing was carried out on a NextSeq sequencer, in $2 \times 150$-bp mode, generating 3,318,986 reads. The raw reads were trimmed using Trimmomatic v0.36 (9).

All three genome sequences were assembled in Unicycler v0.4.8 (10) using long- and short-read sequences in hybrid assembly mode with standard parameters. The assembly resulted in one circular contig for isolates 12-05460 and 19-05816, representing their chromosomes, and two circular contigs, representing a chromosome and a plasmid, for isolate 18-04415. The genome sequences were rotated to the replication origin, and correct ring closure was confirmed by remapping the raw reads. The assembly statistics were calculated in QUAST v5.0.0 (11). The closed genome sequence of 12-05460 had a sequencing depth of 123-fold, a length of 2,986,724 bp, and a GC content of 37.95%. The genome sequence of 19-05816 had a sequencing depth of 165-fold, a length of 2,951,169 bp, and a GC content of 37.96%. The coverage for 18-04415 was 140-fold, and the genome was 2,951,919 bp long, with a GC content of 37.96%. This strain also contained the 86.6-kb plasmid pLMN1546 previously found in a Swiss outbreak strain (12). All sequences were submitted to NCBI and annotated using the NCBI Prokaryotic Genome Annotation Pipeline (13).

**Data availability.** The raw sequencing data and complete genome sequences are available at NCBI and ENA. All accession numbers can be found in Table 1.

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