Francisella tularensis subsp. mediasiatica is the least studied among the four F. tularensis subspecies. We present here the genome data of F. tularensis subsp. mediasiatica 240, isolated in the southern region of Kazakhstan.

Tularemia is a zoonotic natural focal infection caused by Francisella tularensis. Currently, four subspecies of F. tularensis are recognized, differing in virulence and geographical distribution. F. tularensis subsp. tularensis (type A) is common in North America. It is the most virulent subspecies for humans. The two subtypes A.I and A.II also differ in virulence (1). F. tularensis subsp. holarctica is the second most virulent for humans and is distributed in the Northern Hemisphere (2). F. tularensis subsp. novicida, described in North America and Australia, causes sporadic opportunistic infections in immunosuppressed patients (3, 4). F. tularensis subsp. mediasiatica remains the least-studied subspecies. For a long time, it was assumed that its distribution area was limited to Central Asia (Kazakhstan and Turkmenistan), but it was recently recovered in southern Siberia (5). No human infection caused by F. tularensis subsp. mediasiatica has been reported so far. Experiments on model animals indicate a virulence of F. tularensis subsp. mediasiatica intermediate between that of F. tularensis subsp. tularensis and F. tularensis subsp. holarctica (5).

The genetic diversity of F. tularensis subsp. mediasiatica is poorly known. In this article, we present the genome sequence of strain F. tularensis subsp. mediasiatica 240, isolated in 1982 from ticks in the southern region of Kazakhstan. The strain was isolated using direct plating of homogenized sample onto coagulated chicken egg yolk. The inoculations were kept under aerobic conditions at 37°C for 120 h, and typical colonies were subjected to reseeding and further typing. After identification, the strain was stored in a lyophilized state. Before the study, the lyophilized strain was suspended in 0.9% NaCl, plated onto petri dishes with FT agar including vitamins and mineral additives (FBIS SRCAMB, Obolensk, Russia) (5, 6), and cultured under aerobic conditions at 37°C for 72 h. A single colony was subcultured on a petri dish with FT agar and incubated at 37°C for 72 h. The bacterial mass was collected and suspended in 0.9% NaCl. The bacterial suspension was inactivated by adding a thimerosal solution (T5125, Sigma-Aldrich) to a concentration of 0.01% and incubated at 56°C for 30 min.

DNA was isolated using a DNA minikit (Qiagen, Hilden, Germany). Preparation of the sequencing libraries was carried out using the Nextera XT DNA library prep kit (Illumina, San Diego, CA, USA). Sequencing was performed using the MiSeq system with the MiSeq reagent kit v3 (600 cycles, 2 × 300 bp). In total, 780,074 sequencing reads were obtained. The reads were trimmed using Seqtv v1.3 (7) up to a quality (Q) value of Q30 and de novo assembled with Skesa v2.3.0 (8) (all software was used
FIG 1  Maximum parsimony tree of whole-genome single nucleotide polymorphism (SNP) data. Whole-genome sequencing (WGS) data from all available F. tularensis subsp. mediasiatica strains, including Kazakhstan strain 240 (indicated in red), and from selected strains representing the main sublineages of F. tularensis subsp. tularensis and F. tularensis subsp. holarctica were mapped onto reference genome SCHU S4 (assembly accession no. GCA_000008985.1), as previously described (5). In total, 10,953 SNPs were called; the tree size is 11,027 bp (homoplasy, 0.67%). Branch length of the longer branches is indicated. Branches of 70 and 44 SNPs lead to lineages M.I and M.II (Siberian strains), respectively. The red star indicates the branching point toward F. tularensis subsp. novicida, which can be considered an outgroup with respect to the three other subspecies.
sis and Francisella novicida. Front Cell Infect Microbiol 4:35. https://doi.org/10.3389/fcimb.2014.00035.

4. Samrakandi MM, Zhang C, Zhang M, Nietfeldt J, Kim J, Iwen PC, Olson ME, Fey PD, Duhamel GE, Hinrichs SH, Cirillo JD, Benson AK. 2004. Genome diversity among regional populations of Francisella tularensis subspecies tularensis and Francisella tularensis subspecies holarctica isolated from the US. FEMS Microbiol Lett 237:9–17. https://doi.org/10.1016/j.femsle.2004.06.008.

5. Timofeev V, Bakhteeva I, Titareva G, Kopylov P, Christiany D, Mokrievich A, Dyatlov I, Vergnaud G. 2017. Russian isolates enlarge the known geographic diversity of Francisella tularensis subsp. mediiasiatica. PLoS One 12:e0183714. https://doi.org/10.1371/journal.pone.0183714.

6. Morozova TP, Domotenko LV, Khramov MV. 2010. Evaluation of the diagnostic properties of transparent nutrient medium for cultivation and isolation of tularemia agent (Ft-agar). Probl Osobo Opas Infekc 105:50–53. https://doi.org/10.21055/0370-1069-2010-3(105)-50-53.

7. Li H. 2012. seqtk toolkit for processing sequences in FASTA/Q formats. https://github.com/lh3/seqtk.

8. Souvorov A, Agarwala R, Lipman DJ. 2018. SKESA: strategic k-mer extension for scrupulous assemblies. Genome Biol 19:153. https://doi.org/10.1186/s13059-018-1540-z.

9. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.

10. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.

11. Zhao Y, Wu J, Yang J, Sun S, Xiao J, Yu J. 2012. PGAP: pan-genomes analysis pipeline. Bioinformatics 28:416–418. https://doi.org/10.1093/bioinformatics/bts655.