



Whole-Genome Sequences of Clinical *Enterobacter bugandensis* Isolates from Germany

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ABSTRACT *Enterobacter bugandensis* is the most pathogenic species of the genus *Enterobacter* and is a cause of life-threatening infections in neonates. Curiously, it was also detected in samples from the International Space Station. Here, we present complete closed genome sequences of two clinical *E. bugandensis* isolates recognized for the first time in Germany.

Infections with *Enterobacter* spp. are notoriously difficult to treat, and broad resistance to third-generation cephalosporins and quinolones is an increasing problem (1). *Enterobacter* species are members of the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) group of pathogens and are on the WHO priority list for developing new antimicrobials (2). Members of this genus are ubiquitous in nature, but some species are opportunistic human pathogens, particularly those causing neonatal infections and infections associated with the urinary tract, respiratory tract, and bloodstream. Recently, a novel species, *Enterobacter bugandensis*, was identified from a neonatal outbreak in Tanzania with a 35% case fatality rate, and it is currently considered the most pathogenic member of this genus (1, 3). Since the original description, *E. bugandensis* was only reported from the International Space Station as a multidrug-resistant species persisting under microgravity conditions (4). Here, we report the first recognition of *E. bugandensis* involved in clinical cases in Germany.

Two isolates, F-1367 and Survcare220, were recovered on 5% sheep blood agar from the blood culture of a neonatal infection and the throat swab of an 83-year-old patient, respectively. Routine species identification using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) (Vitek) and the Vitek 2 Gram-negative (GN) identification (ID) card (bioMérieux, France) classified them as *Enterobacter* spp. but did not permit species allocation.

For both isolates, complete genome sequences were generated by combining long reads from Oxford Nanopore Technology (ONT) and short reads from Illumina technology. In brief, DNA was isolated from overnight cultures in LB broth using a PureLink genomic DNA kit (Thermo Fisher, Germany). For long reads, libraries were prepared with a native barcoding kit (EXP-NBD103) and 1D chemistry (SQK-LSK108). Sequencing was carried out on a MinION instrument, using a SpotON flow cell Mk I R9 version (FLO-MIN106). For bioinformatic analysis, default parameters were used for all software

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unless otherwise specified. Long-read demultiplexing was performed using Porechop (<https://github.com/rwick/Porechop>). For short reads, libraries were prepared with a Nextera XT library preparation kit (Illumina, Netherlands) and sequenced on NextSeq 500 (midoutput kit v2, 2 × 150 bp) and MiSeq (MiSeq v3 chemistry, 2 × 300 bp) instruments, respectively.

Using ONT, a total of 1,463,879 reads (average read length [ARL], 1,307 nucleotides [nt]; N_{50} , 2,416 nt) were obtained for isolate F-1367. For Survcare220, a total of 1,064,560 reads (ARL, 970 nt; N_{50} , 1,417 nt) were obtained. The total number of short reads was 7,221,934 (ARL, 132 nt) for F-137 and 1,168,524 (ARL, 272 nt) for Survcare220. Hybrid genome assembly was carried out by Unicycler v0.4.6 combining long reads and Trimmomatic v0.36 filtered short reads (5, 6). For F-1367, assembly resulted in only one closed contig of 4,750,456 bp with a GC content of 55.97%. In the case of Survcare220, two circularized contigs of 4,766,761 bp (chromosome, 55.08% GC content) and 151,140 bp (52.05% GC content) resulted. Use of pMLST v2.0 identified a 151,140-bp contig with the IncFIB/IncFIA replicons that was classified as a plasmid. The final (filtered Illumina short-read and ONT long-read) coverages for the genome of F-1367 and Survcare220 were estimated to be 281- and 124- fold, respectively.

Both isolates exhibited an average nucleotide identity of 98.8% to *E. bugandensis* EB-247^T (GenBank accession number [NZ_LT992502](https://www.ncbi.nlm.nih.gov/nuclseq/NZ_LT992502)), as determined by JSpecies v1.2.1 using the “blastall” option. The *in silico* DNA-DNA hybridization score (*isDDH*) determined by the GGDC v2.1 tool was 89%, thus identifying the isolates as *E. bugandensis*. Since the initial discovery of *E. bugandensis* in 2011, this is the first time that this species has been isolated from clinical samples outside Africa. We note that unambiguous assignment is currently possible only by using whole-genome sequencing, and there is a need for rapid detection methods, e.g., using MALDI-TOF. The *Enterobacter cloacae* Multilocus Sequence Typing scheme database (<https://pubmlst.org/ecloacae/>, accessed 20 March 2019; powered by BIGSdb v1.22.3), which is comprised of different *Enterobacter* species, assigned F-1367 and Survcare220 to sequence type 795 (ST795) and the novel ST1140, respectively.

As of May 2019, only one other complete genome sequence of *E. bugandensis* is available in public databases. The complete genome sequences of these two new isolates will facilitate comparative genomic studies to identify genomic variations and describe the diversity of the pangenome of this highly pathogenic species.

Data availability. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number [PRJNA531146](https://www.ncbi.nlm.nih.gov/nuclseq/PRJNA531146). For *E. bugandensis* F-1367, Survcare220, and pSurvcare220, the accession numbers are [CP039452](https://www.ncbi.nlm.nih.gov/nuclseq/CP039452), [CP039453](https://www.ncbi.nlm.nih.gov/nuclseq/CP039453), and [CP039454](https://www.ncbi.nlm.nih.gov/nuclseq/CP039454), respectively, and the versions described in this paper are the first versions. Sequence read data for the whole genome of *E. bugandensis* F-1367 and Survcare220 have been deposited in the Sequence Read Archive under the accession numbers [SRR8858675](https://www.ncbi.nlm.nih.gov/sra/SRR8858675) and [SRR8858676](https://www.ncbi.nlm.nih.gov/sra/SRR8858676), respectively.

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