First Complete Genome Sequence of *Brucella abortus* 2308 isolated from an abortion storm in a dairy farm in India

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Short Report

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Abstract

The present report communicates the first complete genome sequence of *Brucella abortus* 2308 strain isolated from a an abortion storm in a dairy farm located at Kanpur, Uttar Pradesh in India. It caused the last trimester abortions of 32 animals out of 100 cows in a dairy over a period of 60 days. The bacteria were isolated in pure culture from the placenta of aborted cows. The genome sequence length of isolated bacteria is 3,285,606 bp with a 57.25 % GC content, an N₅₀ value of 296,426, L50 value of 4 containing 3,119 coding DNA sequences (CDSs), 49 tRNAs, 1 transfer messenger RNA (mRNA), and 3 rRNA genes. It is the first report of *Brucella abortus* 2308 isolation and complete genome sequence from Indian subcontinent.

Main Text

Brucellosis is endemic in Indian subcontinent and causes severe economic losses to dairy industry due to post abortion syndrome like loss of production, retention of placenta, repeat breeding and ultimately culling of infected animals (Kumar et al., 2019). There is no treatment recommended for *Brucella* positive animals, these are culled and sacrificed as per the guidelines of world Animal Health Organization (OIE Terrestrial Manual, 2004). However, in India due to mythological reasons *Brucella* positive cattle are not sacrificed and maintained in isolation (Kumar et al., 2018). These infected animals might be a source of zoonotic infections as major source of human brucellosis is animal to human contact and *Brucella abortus* is second in the list of zoonotic *Brucella* species following the *Brucella melitensis*, that is transmitted from goats and sheep (Gupta et al., 2014). Out of different strains of *Brucella abortus*, strain 2308 is considered as the most pathogenic strain for human (Suárez-Esquível et al., 2016). It is the first report of *Brucella abortus* 2308 from the country. There are published reports from the different regions of India suggesting the endemicity of brucellosis in country (Kumar et al., 2016). In such scenario, the isolation of *Brucella abortus*, strain 2308 from the clinical cases of abortions in dairy farm, is of major concern. Considering the importance of Brucellosis prevention and control, Government of India launched National Animal Disease Control Programme (NADCP) in 2019 with the target to eradicate brucellosis by 2030. This programme includes calf hood vaccination of female calves with *Brucella abortus* strain 19 vaccine. This makes, it essential to get the complete genome sequence of clinical pathogenic isolate to compare it from the vaccine strain. The comparison will be useful for establishing the efficacy of vaccine and also for the development of diagnostics to differentiate between infected and vaccinated animals as in India, infected animals are not sacrificed. The availability of whole genome sequence of field isolate can be used to study its virulence attributes, pathogenicity markers, host pathogen interaction, phylogenetic relationships with existing pathogenic strains, antigenicity and immunogenicity of different proteins to ascertain potential vaccine and diagnostic targets for better prevention and control of brucellosis. Based on these concepts the present study reports first whole genome sequence of *Brucella abortus* 2308 isolated from the clinical cases of abortions in dairy cattle in India. It was isolated from the placenta of a cow aborted in the eight month of pregnancy in a dairy farm located at Kanpur city of Uttar Pradesh, India. The placenta was collected aseptically, sealed in sterile collection bag and transported to

laboratory overnight. The placenta fluid was streaked over multiple *Brucella* selective media agar plates (HiMedia, India) and incubated at 37°C in the presence of 5% CO₂. The plates were observed for bacterial growth, until the growth observed on the fifth day of inoculation. It was subjected to cultural, morphological and biochemical characterization (Carbol 1997). Bacterial DNA was extracted using phenol chloroform method (Sambrook and Russel, 2001) and DNA was checked using agarose gel electrophoresis, and the purity and quantity were estimated using a spectrophotometer. *Brucella abortus* omp28 gene was amplified by PCR with previously described primers (Lim *et al.*, 2012). Amplicon was subjected for cloning and sequencing for the confirmation of *Brucella abortus*. The pure culture is maintained over *Brucella* selective agar slants. For whole genome sequencing, a single colony was picked to extract genomic DNA with GeneJET genomic DNA purification kit (Thermo Fisher Scientific) and subjected to construct Genomic DNA libraries for sequencing. Each library was sequenced to produce 100 bp paired end reads on an Illumina HiSeq2000 by Biokart India Pvt. Ltd, (Bengaluru, India) to achieve an average of 25× coverage per sample (ranging from 14 to 48×). Primary FASTQ files have been submitted to SRA, BioProject PRJNA610713. A total of 12,561,512 raw data reads were generated with a average length of 149 (nt), and a total of 12,560,987 clean reads were obtained with a average length of 149 (nt) using CLC Genomic workbench 7.0.4 to trim off Illumina adaptor sequences and removing low-quality reads. A total of 1872254445 reads passed the quality filter; reads averaged 152 bp in length and showed an average quality score above Q30 in more than 90% of the bases. Sequences were assembled using SPAdes version 3.11.0 (16) into 30 contigs at least 200 nucleotides (nt) long with largest contig of 609,417 bp and a coverage of >10X, for a total of 3,278,307 bp with a GC content of 57.25%, an N50 value of 296,426, and an L50 value of 4 and containing 3,147 coding DNA sequences (CDSs), 48 contigs, 48 scaffolds, 30,901 ORFs and 3330 genes with average length of 853.828 (nt) 51 tRNAs, 1 transfer messenger RNA (tmRNA), and 3 rRNA genes as identified by annotation using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) version 4.11 (Tatusova *et al.* 2016) and RAST version 2.0 (Aziz *et al.* 2008). The bacterial genome a sequence has been deposited in NCBI GenBank under the accession number JABBV0000000000.

Declarations

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Conflicts of interest/Competing interests

All authors declare that they have no conflict of interest.

Authors' Contributions

Amit Kumar, conducted the basic isolation and Surendra Uphadhyay, Anamika Bhordia and Vinod Kumar Singh performed the cultural, morphological and molecular characterization of the isolates. Malyaj R Prajapati and Jitender Singh performed all the desired bioinformatics analysis. The original manuscript was prepared by Amit Kumar and edited/ reviewed by Anu Rahal, Pankaj Kumar, Ravindra Kumar and Anil Sirohi.

Ethics approval

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted on placenta collected from the aborted animals.

Consent to participate

Not applicable.

Consent for publication

All authors give consent for publication.

Data availability

This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the Bioproject number PRJNA610713 and accession number JABBV000000000. The sequences have been submitted to the Sequence Read Archive (SRA) under the accession number [SRR11526759](#). The version described in this paper is version JABBV000000000.

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