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Genome Note

Genomic analysis of a multidrug-resistant *Brucella anthropi* strain isolated from a 4-day-old neonatal sepsis patient

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ABSTRACT

Objectives: *Brucella anthropi* is a Gram-negative, aerobic, motile, oxidase-positive, non-fermentative Alphaproteobacteria belonging to the family Brucellaceae. It is most commonly found in soil but is an emerging, opportunistic, nosocomial human pathogen. The objective of this study was to understand the genome features of a drug-resistant *B. anthropi* (SOA01) isolated from a blood culture of a 4-day-old neonate and to determine its antimicrobial resistance and pathogenic potential.

Methods: Hybrid genome assembly of *B. anthropi* strain SOA01 was generated using quality-trimmed short Illumina and long MinION reads. Identification and antimicrobial susceptibility profile were determined by MALDI-TOF, in silico ribosomal multilocus sequence typing (rMLST) and VITEK®2, respectively. PATRIC webserver and VFDB were used to identify antimicrobial resistance (AMR), virulence factor (VF) and transporter genes.

Results: Multidrug-resistant *B. anthropi* strain SOA01 has a genome of 4 975 830 bp with a G+C content of 56.29%. Several AMR, VF and transporter genes were identified in the genome. Antimicrobial susceptibility testing revealed resistance to different classes of antibiotics in strain SOA01.

Conclusion: *Brucella anthropi* SOA01 is a multidrug-resistant strain. Several AMR and VF genes were identified in the genome, revealing the potential threat posed by this pathogen. The genome data generated in this study are likely to be useful in better understanding its AMR mechanisms, pathogenic potential and successful adaptation from its primary habitat of soil to the human system. Since it is often misidentified as *Brucella melitensis* or *Brucella suis*, genome characterisation and detailed understanding of its biology are crucial.

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Introduction

Brucella anthropi, until recently known as *Ochrobactrum anthropi*, is a rod-shaped, non-fermentative, oxidase-positive, obligately aerobic, soil-dwelling Alphaproteobacteria. It is also known to colonise various organisms, including humans [1]. Recent reports suggest the emergence of *B. anthropi* as an opportunistic nosocomial pathogen [2]. *Brucella anthropi* has been reported to be linked to the use of indwelling medical devices such as intraperitoneal catheters, central venous catheters and drainage tubes [3,4]. It has been widely reported to cause infective endocarditis, endophthalmitis, meningitis, osteochondritis, urinary tract infection, osteomyelitis, etc. [5]. Owing to the difficulty in identification of this bacterium from other *Brucella* species with similar phenotypes, i.e. *Brucella melitensis* and *Brucella suis*, the clinical relevance of *B. anthropi* infections is yet to be accurately described.

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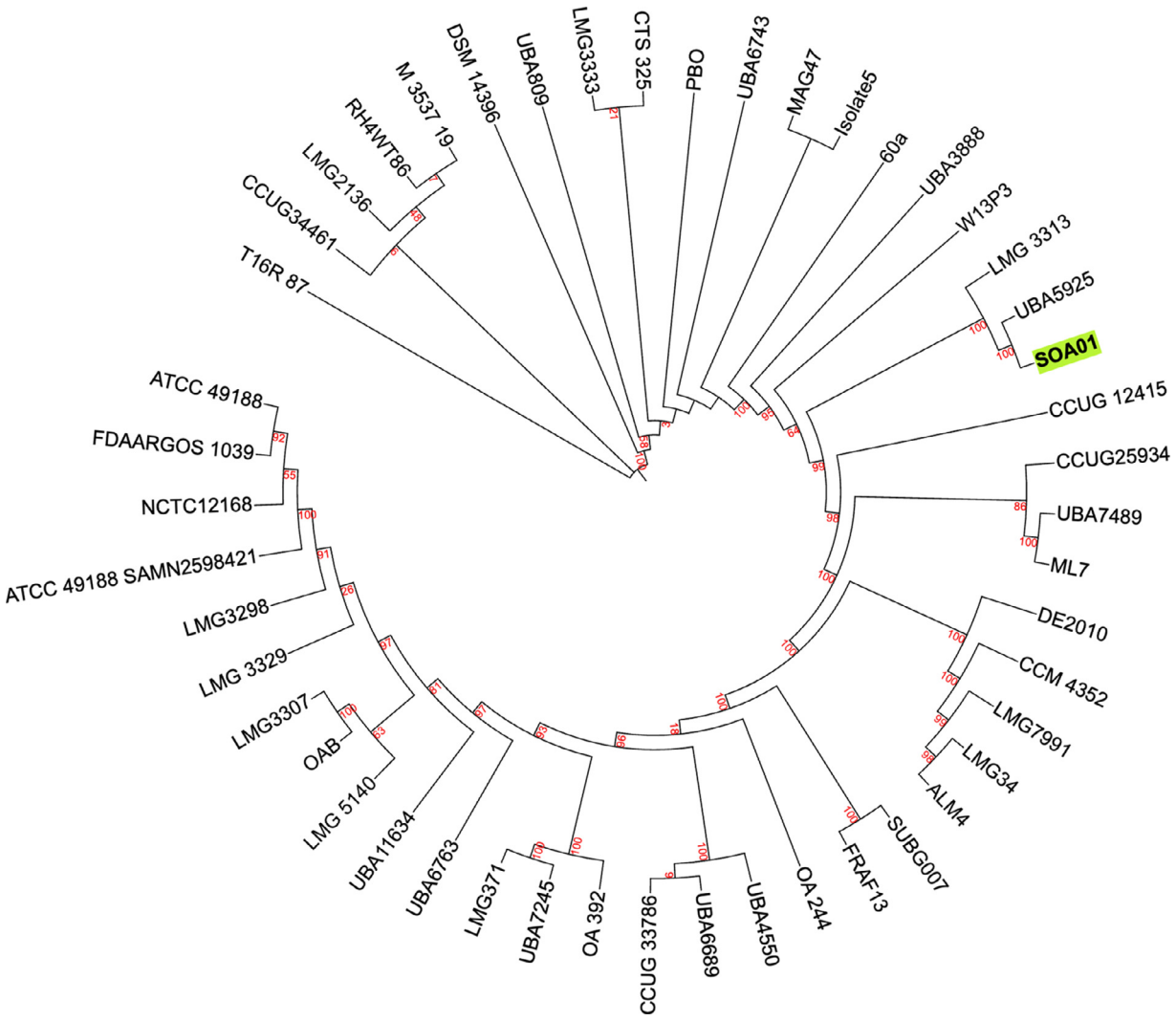

Fig. 1. Core genome phylogeny of *Brucella anthropi* strain SOA01. Roary v.3.13.0 was used to generate the core gene alignment of 48 *B. anthropi* genomes. The alignment file was used as input for the construction of a tree based on the neighbour-joining method using the PHYLIP program. Bootstrap values were scaled from 0–100. The phylogenetic tree was further visualised in iTOL v.6 (https://itol.embl.de). The genome of strain SOA01 (highlighted) clustered together with UBA5925 (metagenomic assembly) and LMG 3313 (isolated from a human vaginal tract).

Materials and methods

Brucella anthropi strain SOA01 was isolated in 2018 from a blood culture of a 4-day-old infant at Sri Sathya Sai General Hospital in Puttaparthi, Andhra Pradesh, India. The newborn was diagnosed with neonatal sepsis. VITEK®2, matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) and in silico ribosomal multilocus sequence typing (rMLST) were used for the identification of bacterial species. Antimicrobial susceptibility testing was performed using VITEK®2 (AST-N281 card). Minimum inhibitory concentrations (MICs) were interpreted based on Clinical and Laboratory Standards Institute (CLSI) guidelines. The isolate was tested against the following antimicrobial agents: trimethoprim/sulfamethoxazole; nitrofurantoin; tigecycline; ciprofloxacin; nalidixic acid; gentamicin; amikacin; meropenem; imipenem; cefepime; cefoperazone/sulbactam; ceftriaxone; cefuroxime axetil; cefuroxime; piperacillin/tazobactam; amoxicillin/clavulanic acid; and ampicillin. The bacterial isolate was cultured overnight in LB medium at 37°C, and extraction of total genomic DNA was carried out using a NucleoSpin® DNA Purification Kit (Macherey-Nagel) according to the manufacturer's instructions. DNA concentration and purity were estimated using a Qubit® 2 fluorometer and 0.8% agarose gel electrophoresis, respectively. Whole-genome sequencing (WGS) was performed using Illumina HiSeq and Oxford Nanopore MinION platforms. FastQC and MultiQC tools were used to assess the quality of raw sequence data. Illumina reads were processed using fastp to remove low-quality bases and adapter sequences. Unicycler was used for de novo hybrid assembly of quality-trimmed short Illumina reads and long MinION reads. Genome annotation was generated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.5.0 server. PATRIC webserver and the Virulence Factor Database (VFDB) were used to identify antimicrobial resistance (AMR), virulence factor (VF) and transporter genes. IslandViewer 4, PHASTER and ICEfinder were used to identify genomic islands (GIs), prophages, and integrative and conjugative elements (ICEs), respectively. PHYLIP and Roary v.3.13.0 were used for construction of the core genome phylogenetic tree.

Results and discussion

The genome sequence of *B. anthropi* strain SOA01 was found to contain six contigs with a total size of 4 975 830 bp. NCBI-PGAP predicted 4582 protein-coding sequences, 8 rRNAs (two 5S rRNA and three each of 16S and 23S rRNAs), 53 tRNAs, 4 ncR-

NAs and 106 pseudogenes. Core genome analysis involving 48 *B. anthropi* genomes revealed that SOA01 was found to cluster with *B. anthropi* UBA5925 (metagenome assembly) and LMG 3313 (human vaginal tract) (Fig. 1). Results of antimicrobial susceptibility testing using VITEK®2 suggested that strain SOA01 exhibited resistance to ampicillin (≥32 μg/mL), amoxicillin/clavulanic acid (≥32 μg/mL), piperacillin/tazobactam (≥128 μg/mL), cefuroxime (≥64 μg/mL), cefuroxime axetil (≥64 μg/mL), ceftriaxone (≥64 μg/mL), cefoperazone/sulbactam (≥64 μg/mL), cefepime (32 μg/mL), imipenem (≥16 μg/mL), meropenem (≥16 μg/mL), nitrofurantoin (256 μg/mL) and trimethoprim/sulfamethoxazole (160 μg/mL). However, the isolate was susceptible to amikacin (16 μg/mL), gentamicin (2 μg/mL), nalidixic acid (16 μg/mL), ciprofloxacin (1 μg/mL) and tigecycline (1 μg/mL). Analysis of mobile genetic elements revealed the presence of 20 GIs, 2 putative ICEs and 3 prophage regions (2 incomplete and 1 questionable). No plasmid sequences were detected in the genome. A total of 44 genes associated with multiple mechanisms of antibiotic resistance were identified (Supplementary Table S1), including genes encoding enzymes that inactivate antibiotics or modify antibiotic targets, efflux pump-encoding genes, genes responsible for alteration of cell wall surface charge, as well as regulatory factors involved in the expression of AMR genes and those that have mutations leading to antibiotic resistance. Furthermore, VFs responsible for immune evasion, intracellular survival, iron uptake, two-component regulatory system and type IV secretory system found in the study genome were compared with nine genomes of other *Brucella* spp. (Supplementary Table S2). Additionally, genes encoding ATP-binding cassette (ABC) superfamily and major facilitator superfamily (MFS) transporters were predicted in the study genome.

Brucella anthropi is an emerging, uncommon, opportunistic, nosocomial pathogen. It is usually misidentified with other closely related *Brucella* species, hence WGS is necessary for accurate identification. Furthermore, understanding of the clinical relevance and pathogenic potential of *B. anthropi* more specifically among neonates requires additional investigations. In this regard, the genome data generated from this study may aid in gaining insights into the mechanisms involved in the emergence of multidrug resistance and the acquisition of AMR genes in this underreported pathogen. Given that the pathogen is primarily a soil-dwelling organism, the genome data may aid in understanding its successful adaptation to emerge as a human pathogen.

Nucleotide sequence accession no

The draft genome sequence and annotation data of *B. anthropi* strain SOA01 described here can be freely and openly accessed in the NCBI database under the accession no. **JAFEHG000000000**. The BioProject and BioSample numbers are **PRJNA698411** and **SAMN17717947**, respectively.

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Competing interests:

None declared.

Ethical approval:

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.06.013.

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