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Genome Note

Genomic and phylogenetic analysis of a multidrug-resistant *Burkholderia contaminans* strain isolated from a patient with ocular infection

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ABSTRACT

Objectives: The genus *Burkholderia* comprises rod-shaped, non-spore-forming, obligately aerobic Gram-negative bacteria that is found across diverse ecological niches. *Burkholderia contaminans*, an emerging pathogen associated with cystic fibrosis, is frequently isolated from contaminated medical devices in hospital settings. The aim of this study was to understand the genomic characteristics, antimicrobial resistance profile and virulence determinants of *B. contaminans* strain SBC01 isolated from the eye of a patient hit by a cow's tail.

Methods: A hybrid sequence of isolate SBC01 was generated using Illumina HiSeq and Oxford Nanopore Technology platforms. Unicycler was used to assemble the hybrid genomic sequence. The draft genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline. Antimicrobial susceptibility testing was performed by VITEK®2. Antimicrobial resistance and virulence genes were identified using validated bioinformatics tools.

Results: The assembled genome size is 8 841 722 bp with a G+C content of 66.33% distributed in 19 contigs. Strain SBC01 was found to possess several antimicrobial resistance and efflux pump genes. The isolate was susceptible to tetracyclines, meropenem and ceftazidime. Many genes encoding potential virulence factors were identified.

Conclusion: *Burkholderia contaminans* SBC01 belonging to sequence type 482 (ST482) is a multidrug-resistant strain containing diverse antimicrobial resistance genes, revealing the risks associated with infections by new *Burkholderia* spp. The large G+C-rich genome has a myriad of virulence factors, highlighting its pathogenic potential. Thus, while providing insights into the antimicrobial resistance and virulence potential of this uncommon species, the present analysis will aid in understanding the evolution and speciation in the *Burkholderia* genus.

The genus *Burkholderia* comprises obligately aerobic, non-spore-forming, rod-shaped, motile Gram-negative bacteria. Members of this genus are common soil inhabitants [1] and have also been identified in water, sewage and animals [2]. *Burkholderia contaminans* was included as a member of the *Burkholderia cepacia* complex group in 2009. In recent years, *B. contaminans* has been epidemiologically linked to contamination of aqueous pharmaceutical products, intravenous fentanyl solution and indwelling medical devices and is widely recognised as an opportunistic nosocomial pathogen [3]. It is considered as an emerging pathogen involved in several outbreaks across different geographic locations. In cystic fibrosis patients, *B. contaminans* infection has been implicated in

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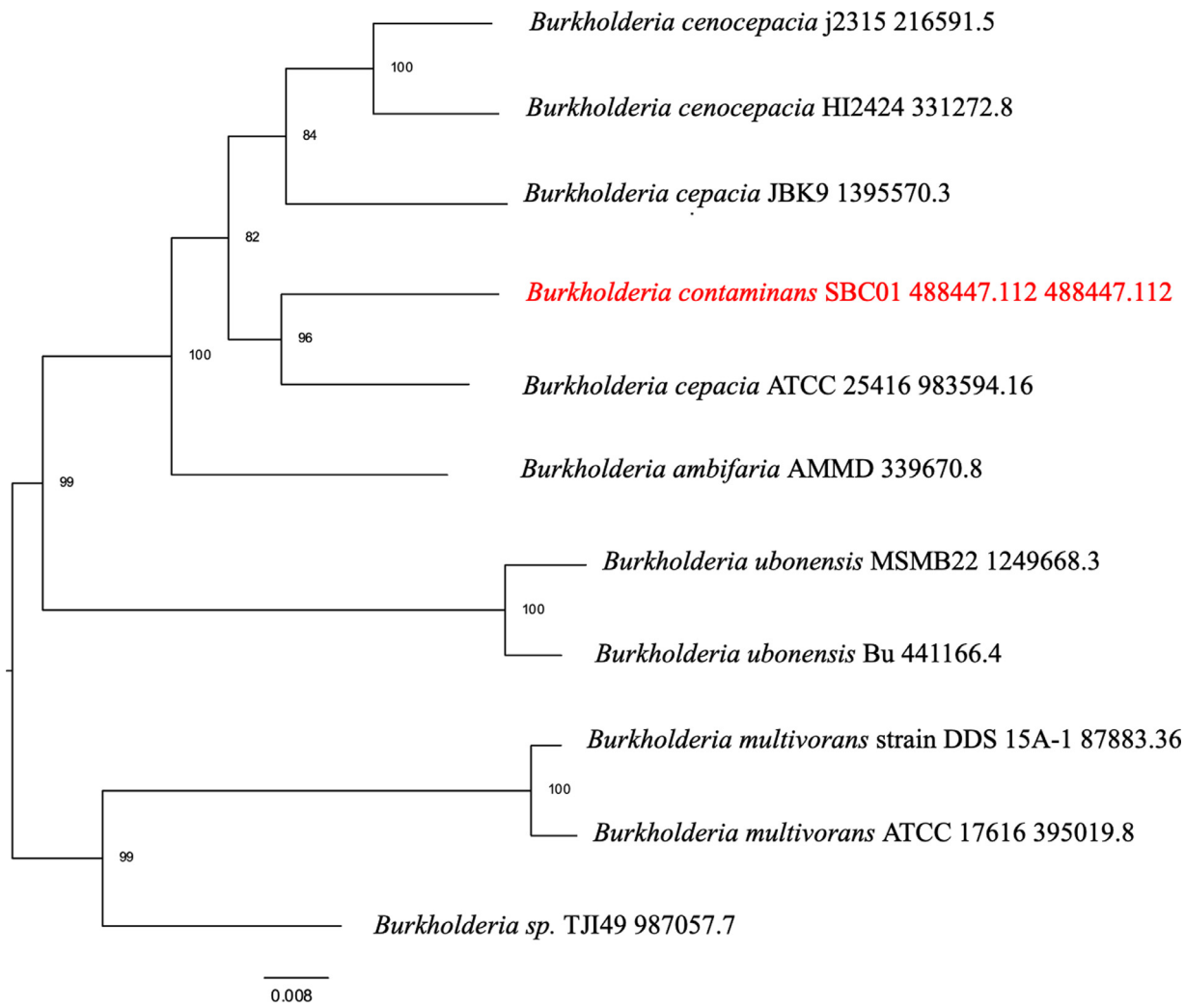

Fig. 1. Phylogenetic tree of *Burkholderia contaminans* strain SBC01 relative to representative strains of other *Burkholderia* species. The phylogenetic tree was generated using the codon tree method within PATRIC, which selected PGFams as homology groups and determined the phylogenetic placement of this genome. The alignment of protein sequences was performed using MUSCLE, and the data matrix of the set of amino acid and nucleotide alignments were analysed using RaxML, with fast bootstrapping.

'cepacia syndrome', leading to rapid decline in lung function and necrotizing pneumonia [4]. A striking element of *B. contaminans* is its capacity to produce antifungal compounds and to thrive in a polymicrobial environment [5]. The aim of this study was to undertake genome sequencing and analysis of a new *B. contaminans* strain (SBC01) isolated from the site of an ocular infection to understand its antimicrobial resistance profile and pathogenic potential.

After being hit by a cow's tail in the right eye, the subject experienced redness and pain for nearly 20 days. Further clinical investigation revealed the presence of a fungal ulcer in the infected right eye.

Culture of corneal scrapings were found to be positive for a Gram-negative bacterium in addition to a fungus. Identification of the co-infecting fungus was not attempted. Initially, the bacterium was reported as *B. cepacia* by VITEK®2. However, further analysis by matrix-assisted laser desorption/ionisation time-of-flight mass (MALDI-TOF) identified the isolate as *B. contaminans*. Antimicrobial susceptibility testing of the bacterial isolate was performed using VITEK®2 with AST-N281 card (bioMérieux) as per the manufacturer's instructions. *Burkholderia contaminans* strain SBC01 was found to be resistant to the majority of antibiotics tested (β-lactams, third- and fourth-generation cephalosporins, monobactam, carbapenems, aminoglycosides, fluoroquinolones and sulfonamides), except tetracyclines, meropenem and ceftazidime. Multidrug-resistant strain SBC01 was cultured in LB broth and its genomic DNA was extracted using a NucleoSpin^{TM} DNA Purification Kit (Macherey-Nagel) according to the manufacturer's instructions. DNA quality and quantity were assessed by 0.8% agarose gel electrophoresis and Qubit 2.0 fluorometer, respectively. A hybrid genome assembly was generated using Illumina HiSeq and Oxford Nanopore Technology platforms. FastQC and MultiQC tools were used to assess the sequencing quality of the genome. Quality trimming of adapter sequences and low-quality bases generated by the Illumina sequencing platform was performed using fastp. Quality-trimmed reads from Illumina and Nanopore were assembled using Unicycler. The species of the assembled hybrid genome was confirmed by ribosomal multilocus sequence typing (rMLST) analysis. MLST analysis using the PubMLST server revealed that strain SBC01 belonged to sequence type 482 (ST482). BUSCO was used for analysis of the genome's completeness. The draft genome assembly of SBC01 is 8 841 722 bp long with a mean GC content of 66.33%. It has 19 contigs larger than 10 000 bp. The phylogenetic position of the study genome amongst the reference and representative genomes listed in the NCBI database was identified using PATRIC.

Comparison of the study genome with other *Burkholderia* genomes revealed that SBC01 belongs to *B. contaminans* (Fig. 1). The assembled genome was annotated using the NCBI Prokaryotic

Genome Annotation Pipeline (PGAP) v.5.0. A total of 7908 protein-coding sequences, 57 tRNAs, 3 rRNAs, 4 ncRNAs and 120 pseudogenes were predicted in the genome. The antimicrobial resistance genes *adeF* (fluoroquinolones and tetracyclines), *amrA* (aminoglycosides), *tet(D)* (tetracyclines) and *ceoA* (sulfonamides) were identified using the CARD-RGI tool. Notably, 79 genes coding for virulence factors (VFs) were predicted in the study genome using the VF analysis tool of the Virulence Factor Database (VFDB). Among others, VFs belonging to adherence, antiphagocytosis, invasion, biofilm formation, type VI secretion system, colonisation, immune evasion, siderophores, endotoxins and two-component regulatory systems were found in the SBC01 genome. Draft genome data of *B. contaminans* SBC01 offer insights into the antimicrobial resistance profile and virulence trends of this uncommon species and may aid in understanding the evolution and speciation in the *Burkholderia* genus.

Nucleotide sequence accession no.

The draft genome sequence and annotation data of *B. contaminans* SBC01 described here can be freely and openly accessed in the NCBI database under accession no. **JAFEHF000000000**. The BioProject and BioSample numbers are **PRJNA698340** and **SAMN17714623**, respectively.

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Declaration of Competing Interest

None declared.

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Ethical approval

Not required.

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