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Genome Note

Genomic insights into an extensively drug-resistant and hypervirulent *Burkholderia dolosa* N149 isolate of a novel sequence type (ST2237) from a Vietnamese patient hospitalised for stroke



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ABSTRACT

Objectives: *Burkholderia dolosa* is a clinically important opportunistic pathogen in inpatients. Here we characterised an extensively drug-resistant and hypervirulent *B. dolosa* isolate from a patient hospitalised for stroke.

Methods: Resistance to 41 antibiotics was tested with the agar disc diffusion, minimum inhibitory concentration, or broth microdilution method. The complete genome was assembled using short-reads and long-reads and the hybrid de novo assembly method. Allelic profiles obtained by multilocus sequence typing were analysed using the PubMLST database. Antibiotic-resistance and virulence genes were predicted in silico using public databases and the 'baargin' workflow. *B. dolosa* N149 phylogenetic relationships with all available *B. dolosa* strains and *Burkholderia cepacia* complex strains were analysed using the pangenome obtained with Roary.

Results: *B. dolosa* N149 displayed extensive resistance to 31 antibiotics and intermediate resistance to 4 antibiotics. The complete genome included three circular chromosomes (6 338 630 bp in total) and one plasmid (167 591 bp). Genotypic analysis revealed various gene clusters (*acr*, *amr*, *amp*, *emr*, *ade*, *bla* and *tet*) associated with resistance to 35 antibiotic classes. The major intrinsic resistance mechanisms were multidrug efflux pump alterations, inactivation and reduced permeability of targeted antibiotics. Moreover, 91 virulence genes (encoding proteins involved in adherence, formation of capsule, biofilm and colony, motility, phagocytosis inhibition, secretion systems, protease secretion, transmission and quorum sensing) were identified. *B. dolosa* N149 was assigned to a novel sequence type (ST2237) and formed a mono-phylogenetic clade separated from other *B. dolosa* strains.

Conclusions: This study provided insights into the antimicrobial resistance and virulence mechanisms of *B. dolosa*.

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1. Introduction

Burkholderia dolosa belongs to the *Burkholderia cepacia* complex (Bcc) of opportunistic bacteria, and is an important nosocomial pathogen [1,2]. The emergence of multi-resistant *B. dolosa* strains, particularly to the latest generation of antibiotics, is a serious public health problem. This pathogen displays intrinsic resistance to various antibiotics, thus limiting the treatment options

and increasing the mortality rate [3]. The genetic determinants associated with antibiotic resistance in *B. dolosa* have not been extensively investigated. Here, we explored the genomic features of an extensively drug-resistant and hypervirulent *B. dolosa* strain, named N149.

2. Materials and methods

The *B. dolosa* strain N149 was isolated from a bronchial fluid sample of a 80-year-old Vietnamese woman hospitalised for stroke at 108 Military Central Hospital, Hanoi, in 2021. The isolate was identified as *B. dolosa* using the VITEK-MS system (BioMérieux, USA) and confirmed by 16S rRNA sequencing. Antibiotic susceptibility testing (AST) was carried out for 44 antibiotics belonging to 21 antimicrobial classes using the disc diffusion test, the minimum inhibitory concentration (MIC) determined with the VITEK-2 Compact system, or the broth microdilution method. The AST results were interpreted following the 2020 Clinical and Laboratory Standards Institute guidelines.

B. dolosa N149 genomic DNA was extracted using the DNeasy PowerLyzer Microbial Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Short-read whole-genome DNA sequencing was performed at Beijing Genomics Institute (Shenzhen, China) on a BGI-SEQ500 platform using a 2 × 150 paired-end protocol. Long-read sequencing was performed using the Rapid Barcoding Kit (SQK-RBK004) on a R9.4.1 flow cell (FLO-MIN106D) and the MinION M1k device (Oxford, United Kingdom) at University of Science and Technology of Hanoi (USTH), Vietnam. Hybrid *de novo* genome assembly, combining short and long reads, was done with Unicycler v0.4.9, and the completeness was checked with BUSCO v5.2.2.

The genome sequence was annotated using Bakta v1.6.0, and then genotyping was performed according to the multilocus sequence typing (MLST) scheme developed by Institute Pasteur, France. Novel allele profiles were deposited in the PubMLST database. Putative antibiotic resistance genes were predicted *in silico* using a combination of tools, including ResFinder v4.2.3, AMRfinderPlus v3.11.14, Resistance Gene Identifier (RGI) v6.0.2 in the Comprehensive Antibiotic Resistance Database (CARD) v3.2.7. Virulence factors were predicted using VFAnalyzer and the VFDB database (<http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi>). CRISPR-Cas sequences, mobile genetic elements, and prophages were identified with CRISPRCasFinder, MobileElementFinder, and PASTER, respectively. Genomic islands (GIs) were predicted using IslandPick, IslandPath-DIMOB and SIGI-HMM. The core genome-based phylogenetic relationships between *B. dolosa* N149 and the five available *B. dolosa* and 37 representative Bcc strains ($n = 20$ *Burkholderia cepacia*, $n = 2$ *Burkholderia stabilis*, $n = 5$ *Burkholderia vietnamiensis*, $n = 10$ *Burkholderia cenocepacia*) were studied with Roary v3.11.2. The complete circular chromosome maps and phylogeny were visualised with Proksee.

3. Results and discussion

B. dolosa N149 was extensively resistant to 30 drugs belonging to 19 different antibiotic groups, and displayed intermediate resistance to aztreonam, ofloxacin, temocillin and tetracycline. This huge range of antibiotic resistances demonstrates the multi-drug resistant phenotype and challenges the treatment of *B. dolosa* infections. Nonetheless, some second/third generation drugs belonging to groups of aminoglycoside, beta-lactam, cephalosporin, monobactam, particularly fluoroquinolone and tetracycline are still effective against *B. dolosa* infection (Table 1).

B. dolosa N149 complete genome included three circular chromosomes of 3 298 494 bp (chromosome 1), 2 211 974 bp (chromosome 2), and 828 162 bp (chromosome 3), with a GC content from

67.0 to 67.4%, and one plasmid of 167 591 bp with a GC content of 62.2% (Fig. 1A). The genome harboured 5536 known protein-encoding genes, 18 rRNAs, 83 tRNAs and 12 GIs. Mobile genetic elements were found in the GIs, including the prophage integrase *intA*, transposases of the IS3 and IS21 families, and the tyrosine recombinase XerC, which can facilitate the acquisition and transfer of antibiotic-resistance and virulent determinants. Comparative sequence analysis revealed 96% of similarity between the plasmid of *B. dolosa* N149 and the plasmid of *B. cenocepacia* HI2424 (coverage of 57%) (GenBank accession number: CP000461.1), suggesting the potential transmission of plasmids among Bcc via horizontal gene transfers. Interestingly, the completely sequenced plasmid of *B. dolosa* was reported for the first time on NCBI.

B. dolosa N149 resistome comprised various antibiotic-resistance gene clusters (*acr*, *amr*, *emr*, *ade*, *bla* and *tet*) associated with resistance to 35 antibiotic classes, many of which are responsible for resistance to β -lactams, tetracyclines, macrolides, aminoglycosides and fluoroquinolones (Fig. 1A). In concordance with the phenotypic-resistant data, the genotypic-resistant profile highlighted *B. dolosa* N149 multi-drug-resistance. The most common resistance mechanisms implicated alterations in genes encoding efflux pumps, including the resistance-nodulation-cell division, major facilitator superfamily, ATP-binding cassette, multidrug and toxic-compound extrusion, and small multidrug resistance families [4]. Other resistance mechanisms included antibiotic target alteration, antibiotic inactivation, and reduced permeability to antibiotics. These findings underline the important role of intrinsic antibiotic-resistance mechanisms in *B. dolosa* and in Bcc. Furthermore, Bcc species also readily acquire other antibiotic-resistance mechanisms under selective pressure during treatment course. Therefore, genomic surveillance plays a key role in the management and control system of emerging multidrug-resistant pathogens.

B. dolosa N149 carried 79 virulence genes associated with increased adherence to host cells (*boaA*), colony formation, protease secretion and motility (type IV pili), phagocytosis inhibition by formation of capsular and other cell wall components (capsule I *manC*), lipopolysaccharide biosynthesis (*wbjD*), and structural formation of flagella (*che*, *flg*, *flh*, *fli*, *mot* and *tsr* families) (Fig. 1A). Genes encoding proteins related to the secretion systems type II, III, IV and VI, anaerobic respiration (*narG*), biofilm formation (*adeG*), cell surface components (*sugC*), efflux pumps (*acrB*, *farB*), immune evasion (*gtrB*), iron uptake (*pvdF*) and the actin cross-linking toxin VgrG1 were also identified. The PmlI-PmlR quorum sensing system, which contributes to virulence and modulates the MprA protease production in *Burkholderia pseudomallei* [5], was present also in *B. dolosa* N149 genome. The many virulent factors detected in its genome suggest that *B. dolosa* is highly transmissible compared with other Bcc species [6].

Novel alleles in *gltB* (positions 146 A-G and 316 C-T), *gyrB* (101 T-C and 230 T-C) and *atpD* (215 G-A) were detected in *B. dolosa* N149 genome resulting in a novel sequence type (ST2237) based on the Institute Pasteur MLST scheme. Accordingly, in the core genome-based phylogenetic tree, *B. dolosa* N149 was separated from the clade containing other *B. dolosa* strains (Fig. 1B). Furthermore, some Bcc strains were not clustered in the same species clade suggesting that their taxonomic name needs for revision. Presently, the taxonomic relationship of the Bcc species is still not well established [7]. This could be problematic for diagnosis and antimicrobial therapy, and therefore, the accurate classification of Bcc species is particular needed.

In summary, the many antimicrobial resistance and virulence genes found in *B. dolosa* N149 genome could favour its adaptation and survival in various ecological niches. These findings should guide the therapy management and the introduction of measure to control *B. dolosa* spreading.

Table 1
Phenotypic-antibiotic susceptibility test of *B. dolosa* N149.

| No. | Antibiotic group | Antibiotic | Inhibition zone diameter (mm) | AST Result | MIC (µg/mL) |
|-----|--|---|-------------------------------|------------|-------------|
| 1 | Beta-lactam | Ampicillin (10 µg) | 0 | R | |
| 2 | | Penicillin G (1UI) | 0 | R | |
| 3 | | Oxacillin (5 µg) | 0 | R | |
| 4 | Beta-lactam/ Beta-lactamase inhibitor combinations | Temocillin (30 µg) | 20 | I | |
| 5 | | Piperacillin (100 µg) | 23 | S | |
| 6 | | Ticarcillin (75 µg) | 0 | R | |
| 7 | | Amoxicillin/Clavulanic acid (20/10 µg) | 0 | R | |
| 8 | | Ticarcillin/Clavulanic Acid (75/10 µg) | 0 | R | > 128 |
| 9 | | Piperacillin/Tazobactam (100/10 µg) | 32 | S | 32 |
| 10 | | Cephalosporins | Cefotaxime (30 µg) | 0 | R |
| 11 | Cefoxitin (30 µg) | | 0 | R | |
| 12 | Cefepime (30 µg) | | 22 | S | 4 |
| 13 | Carbapenems | Ceftazidime (30 µg) | 30 | S | 8 |
| 14 | | Imipenem (10 µg) | 10 | R | > 128 |
| 15 | | Meropenem (10 µg) | 20 | R | ≥ 16 |
| 16 | | Ertapenem (10 µg) | 10 | R | |
| 17 | Monobactams | Aztreonam (30 µg) | 19 | I | ≥ 16 |
| 18 | Aminoglycosides | Gentamicin (10 µg) | 0 | R | ≥ 16 |
| 19 | | Tobramycin (10 µg) | 0 | R | ≥ 16 |
| 20 | | Amikacin (30 µg) | 10 | R | 64 |
| 21 | Fluoroquinolones | Kanamycin (30 µg) | 20 | S | |
| 22 | | Nalidixic acid (30 µg) | 12 | R | |
| 23 | | Ofloxacin (5 µg) | 14 | I | |
| 24 | | Norfloxacin (10 µg) | 18 | S | |
| 25 | | Ciprofloxacin (5 µg) | 24 | S | ≤ 2 |
| 26 | Folate pathway inhibitors | Levofloxacin (5 µg) | 20 | S | ≤ 2 |
| 27 | | Trimethoprim/sulfamethoxazole (1.25/23.75 µg) | 14 | R | 20 |
| 28 | Polymyxins | Colistin (10 µg) | 0 | R | > 128* |
| 29 | Tetracyclines | Tetracycline (30 µg) | 27 | I | |
| 30 | | Minocycline (30 µg) | 19 | S | |
| 31 | | Tigecycline | ND | ND | ≤ 2 |
| 32 | Macrolides | Erythromycin (15 µg) | 0 | R | |
| 33 | Amphenicols | Chloramphenicol (30 µg) | 10 | R | |
| 34 | Rifamycins | Rifampicin (5 µg) | 0 | R | |
| 35 | Glycopeptides | Vancomycin (5 µg) | 0 | R | |
| 36 | | Teicoplanin (30 µg) | 0 | R | |
| 37 | Fusidanes | Fusidic acid (10 µg) | 0 | R | |
| 38 | Phosphonic acids | Fosfomycin/Trometamol (200UG) | 0 | R | |
| 39 | Oxazolidinones | Linezolid (10 µg) | 0 | R | |
| 40 | Streptogramins | Pristinamycin (15 µg) | 16 | R | |
| 41 | Lincosamides | Clindamycin (2 µg) | 0 | R | |
| 42 | | Lincomycin (15 µg) | 0 | R | |
| 43 | Nitrofurantoin | Nitrofurantoin (100 mg) | 0 | R | |
| 44 | Nitroimidazole | Metronidazole (4µg) | 0 | R | |

NA, not determined.

* The MIC was determined using broth microdilution assay.

Declarations

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Ethical approval: Not required.

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Accession numbers: The complete nucleotide sequences of *B. dolosa* N149 three chromosomes and plasmid were deposited in the NCBI GenBank database with BioProject PRJNA1001802, BioSample SAMN36815367, and were cited as NCBI reference sequences under the following accession numbers: NZ_CP132922.1 (chromosome 1), NZ_CP132923.1 (chromosome 2), NZ_CP132924.1 (chromosome 3), and NZ_CP132925.1 (plasmid).

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