Draft genome sequence of *Acinetobacter baumannii* strain NCTC 13423, a multidrug-resistant clinical isolate

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**Abstract**

*Acinetobacter baumannii* is a pathogen that is becoming increasingly important and causes serious hospital-acquired infections. We sequenced the genome of *A. baumannii* NCTC 13423, a multidrug-resistant strain belonging to the international clone II group, isolated from a human infection in the United Kingdom in 2003. The 3,937,944 bp draft genome has a GC-content of 39.0 % and a total of 3672 predicted protein-coding sequences. The availability of genome sequences of multidrug-resistant *A. baumannii* isolates will fuel comparative genomic studies to help understand the worrying spread of multidrug resistance in this pathogen.

**Keywords:** Draft genome, *Acinetobacter baumannii*, Nosocomial pathogen, Multidrug resistance, Human isolate

**Abbreviations:** COG, Clusters of orthologous groups; PGAP, Prokaryotic genome annotation pipeline

**Introduction**

*Acinetobacter baumannii* recently emerged as an increasingly important pathogen causing healthcare-associated bloodstream, urinary tract, pulmonary, and device-related infections [1]. *A. baumannii* strains are often resistant against multiple antibiotics, owing to their high intrinsic resistance and a variety of acquired resistance mechanisms [2]. Carbapenem is usually an effective treatment choice, but carbapenem-resistant strains are globally on the rise, and alternative treatment options are limited [3].

Here, we present the draft genome sequence of *A. baumannii* NCTC 13423, a strain belonging to international clone lineage II isolated from a patient in a UK hospital in December 2003 [4]. NCTC 13423 shows resistance to ampicillin, amoxicillin-clavulanic acid, aztreonam, cefepime, cefotaxime, cefazidime, cefotixin, piperacillin, piperacillin-tazobactam, ciprofloxacin, gentamicin, and sulbactam [4]. Although originally reported as carbapenem-sensitive, a later report classified it to be also carbapenem-resistant [5]. Additionally, this strain is highly virulent and a strong biofilm producer [6].

**Organism information**

**Classification and features**

Bacteria in the genus *Acinetobacter* are Gram-negative, strictly aerobic, nonfermenting, nonmotile, catalase-positive, oxidase-negative coccobacilli [7] (Table 1). The genus *Acinetobacter* has gone through many taxonomic changes over the years, and the species *A. baumannii* has only been officially recognized since 1986 [8, 9]. *A. baumannii* belongs to the family Moraxellaceae, order Pseudomonadales, class Gammaproteobacteria, and phylum Proteobacteria. *Acinetobacter* species are ubiquitous organisms, widely distributed in nature, and can be recovered from virtually any soil or water sample. However, *A. baumannii* seems to be an exception to this rule, as it currently has no known habitats except the hospital [10]. Microscopically, they are often observed as pairs of cells (Fig. 1). *A. baumannii* can withstand prolonged desiccation, allowing it to survive on dry surfaces and probably contributing to its persistent residence in hospital settings [11]. A phylogenetic tree based on 16S rDNA sequences showed strong clustering with other *A. baumannii* strains (Fig. 2).

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The strain NCTC 13423 was isolated in 2003 in the United Kingdom from a repatriated casualty of the Iraq conflict [4], and was selected for sequencing because of its multidrug-resistant and virulence characteristics. Sequencing was carried out at the EMBL GeneCore facility (Heidelberg, Germany). Sequences were assembled using CLC Genomics Workbench (version 7.5.1) and annotated using NCBI’s Prokaryotic Genome Annotation Pipeline (PGAP). This draft whole-genome sequence has been deposited at DDBJ/ENA/GenBank under the accession LOHD00000000. The project information, and its association with MIGS version 2.0 [12], is summarised in Table 2.

**Growth conditions and genomic DNA preparation**

Cultures for DNA isolation were inoculated from a single colony on LB agar in 5 ml lysogeny broth and grown overnight at 37 °C with orbital shaking (200 rpm). DNA was isolated using the DNeasy Blood&Tissue Kit (Qiagen) following the manufacturer’s instructions and pre-treatment protocol for Gram-negative bacteria. DNA concentration and purity were assessed using the Nanodrop ND-1000 spectrophotometer and Qubit fluorometer (ThermoFisher Scientific).

**Genome sequencing and assembly**

Sequencing was performed using the Nextera DNA Library Preparation Kit with the Illumina HiSeq 2000 platform (100 bp, paired-end) at the EMBL GeneCore facility (Heidelberg, Germany). The read library contained a total of 8,765,016 sequences in pairs. Sequence data was analysed using Qiagen’s CLC Genomics Workbench (version 7.5.1). First, reads were trimmed for quality (score limit 0.05) and ambiguous nucleotides (maximum 2 ambiguities). Next, de novo assembly was performed (mismatch cost: 2, deletion cost: 3, insertion cost: 3, length fraction: 0.5, similarity fraction: 0.8), yielding 196 contigs (minimum length 200 bp) with an average coverage of 203x. Contigs averaged 20,092 bp in length (N50 of 111,328 bp). The total length of the

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**Table 1** Classification and general features of *Acinetobacter baumannii* strain NCTC 13423 according to the MIGS recommendations [12]

| MIGS ID | Property Term | Evidence code a |
|---------|---------------|-----------------|
|         | Domain Bacteria | TAS [29] |
|         | Phylum Proteobacteria | TAS [30] |
|         | Class Gammaproteobacteria | TAS [31, 32] |
|         | Order Pseudomonadales | TAS [33, 34] |
|         | Family Moraxellaceae | TAS [35] |
|         | Genus Acinetobacter | TAS [34, 36] |
|         | Species *Acinetobacter baumannii* | TAS [8] |
|         | Strain NCTC 13423 | NAS |
|         | Gram stain | Negative | TAS [8] |
|         | Cell shape | Cocccobacillus | TAS [8] |
|         | Motility | Non-motile | TAS [37] |
|         | Sporulation | Non-sporulating | TAS [8] |
|         | Temperature range | Mesophilic | TAS [38] |
|         | Optimum temperature | 37 °C | TAS [38] |
|         | pH range; Optimum | Unknown | NAS |
|         | Carbon source | Chemoorganoheterotrophic; citrate, lactate, ethanol, glutarate, malate, aspartate, tyrosine, 2,3-butenediol, 4-aminobutyrate | TAS [8] |
|         | MIGS-6 Habitat | Hospital | NAS |
|         | MIGS-6.3 Salinity | Unknown | NAS |
|         | MIGS-22 Oxygen requirement | Strictly aerobic | TAS [8] |
|         | MIGS-15 Biotic relationship | Free-living | TAS [8] |
|         | MIGS-14 Pathogenicity | Pathogenic | TAS [4] |
|         | MIGS-4 Geographic location | United Kingdom | TAS [4] |
|         | MIGS-5 Sample collection | 12/2003 | TAS [4] |
|         | MIGS-4.1 Latitude | Unknown | NAS |
|         | MIGS-4.2 Longitude | Unknown | NAS |
|         | MIGS-4.4 Altitude | Unknown | NAS |

aEvidence codes, IDA inferred from direct assay, TAS traceable author statement (i.e., a direct report exists in the literature), NAS non-traceable author statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [39].

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**Fig. 1** Phase-contrast micrograph of *A. baumannii* NCTC 13423

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**Genome sequencing information**

**Genome project history**

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draft genome is 3,937,944 bp with a GC-content of 39.0%.

Genome annotation

All contigs were annotated using NCBI’s Prokaryotic Genome Annotation Pipeline (PGAP). The Batch Web CD-Search Tool from NCBI [13] was used to identify Pfam domains [14] in the predicted protein sequences.

Classification of predicted proteins in Clusters of Orthologous Groups (COG) functional categories [15] was done with the WebMGA web server for metagenomic analysis [16]. Signal peptides, transmembrane domains, and CRISPR repeats were predicted using the SignalP 4.1 server [17], the TMHMM server [18], and the CRISPRFinder tool [19], respectively. Only confirmed and not questionable CRISPR hits were taken into account.

Genome properties

Table 3 summarises the properties of the draft genome. Reads were assembled into 196 contigs, totalling 3,937,944 bp with a 39.0 % GC-content. PGAP predicted a total number of 3875 genes, including 3672 protein coding genes (totalling 3,384,768 base pairs), 135 pseudo genes, and 68 RNA genes (64 tRNA, 3 rRNA, and 1 ncRNA). 75.17 % of the protein-coding genes had a putative function assigned, the remainder was annotated as a hypothetical protein. Additional characteristics of the predicted genes are given in Table 3, and Table 4 shows their distribution amongst the different functional COG categories.

Insights from the genome sequence

Functional analysis of the genome sequence by RAST annotation [20] revealed A. baumannii ACICU as the closest related sequenced neighbor. A. baumannii ACICU is an epidemic, multidrug-resistant strain isolated from a hospital.
outbreak in Rome [21]. The high genetic relatedness between A. baumannii ACICU and A. baumannii NCTC 13423 was confirmed by calculating their two-way average amino acid identity (AAI), which was 99.30 % based on 3360 protein sequences [22]. Indicative for the multidrug-resistant phenotype, annotations by RAST included six different β-lactamase enzymes, among which two AmpC-type β-lactamases (class C), a metallo-β-lactamase (class B), two class A β-lactamases (of which one TEM-type broad-spectrum β-lactamase) and an oxa-51 like carbapenemase (class D). Using TAFinder, a web-based tool to identify type II toxin-antitoxin (TA) loci in bacterial genomes [23], we predicted the presence of 12 type II TA modules in the A. baumannii NCTC 13423 draft genome. Considering only TAFinder hits with normalized homology scores (H-value) > 0.5, five putative TA modules remain, three of which are also present in the genome of A. baumannii ACICU. Interestingly, A. baumannii has been reported to form antibiotic-tolerant persister cells [24, 25], and these TA modules might play a role in their formation [26].

Conclusions
We determined the draft genome sequence of the highly virulent, multidrug-resistant A. baumannii NCTC 13423 clinical isolate. The availability of genomic sequences of clinical A. baumannii isolates from a variety of locations and sources will benefit comparative genomic studies to better understand the worrying spread of multidrug resistance in this pathogen.

Table 3 Genome statistics

| Attribute                        | Value    | % of Total |
|----------------------------------|----------|------------|
| Genome size (bp)                 | 3,937,944| 100        |
| DNA coding (bp)                  | 3,384,768| 85.95      |
| DNA G+C (bp)                     | 1,537,664| 39.05      |
| DNA scaffolds                     | 196      | 100        |
| Total genes                      | 3875     | 100        |
| Protein coding genes             | 3672     | 94.76      |
| RNA genes                        | 68       | 1.75       |
| Pseudo genes                     | 135      | 3.48       |
| Genes in internal clusters       | -        | -          |
| Genes with function prediction   | 2913     | 75.17      |
| Genes assigned to COGs           | 3174     | 81.91      |
| Genes with Pfam domains          | 3,002    | 77.47      |
| Genes with signal peptides       | 313      | 8.08       |
| Genes with transmembrane helices | 882      | 22.76      |
| CRISPR repeats                   | 0        | -          |

Table 4 Number of genes associated with general COG functional categories

| Code | Value | %age  | Description                                      |
|------|-------|-------|--------------------------------------------------|
| J    | 177   | 4.82  | Translation, ribosomal structure and biogenesis  |
| A    | 0.03  |       | RNA processing and modification                  |
| K    | 272   | 7.41  | Transcription                                    |
| L    | 125   | 3.40  | Replication, recombination and repair            |
| B    | 0.00  |       | Chromatin structure and dynamics                 |
| D    | 32    | 0.87  | Cell cycle control, Cell division, chromosome partitioning |
| V    | 40    | 1.09  | Defense mechanisms                               |
| T    | 97    | 2.64  | Signal transduction mechanisms                   |
| M    | 193   | 5.26  | Cell wall/membrane biogenesis                    |
| N    | 42    | 1.14  | Cell motility                                    |
| U    | 88    | 2.40  | Intracellular trafficking and secretion          |
| O    | 112   | 3.05  | Posttranslational modification, protein turnover, chaperones |
| C    | 202   | 5.50  | Energy production and conversion                 |
| G    | 138   | 3.76  | Carbohydrate transport and metabolism            |
| E    | 288   | 7.84  | Amino acid transport and metabolism              |
| F    | 81    | 2.21  | Nucleotide transport and metabolism              |
| H    | 131   | 3.57  | Coenzym transport and metabolism                 |
| I    | 182   | 4.96  | Lipid transport and metabolism                   |
| P    | 185   | 5.04  | Inorganic ion transport and metabolism           |
| Q    | 97    | 2.64  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 406   | 11.06 | General function prediction only                 |
| S    | 285   | 7.76  | Function unknown                                 |
| -    | 498   | 13.56 | Not in COGs                                     |

The total is based on the total number of protein coding genes in the genome.

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Authors’ contributions
JEM performed the experiments, analysed the data, and wrote the manuscript. BVDB and MF helped analysing the data and edited the manuscript. JM initiated and supervised the study, and edited the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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