

Acinetobase: the comprehensive database and repository of *Acinetobacter* strains

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Abstract

Acinetobacter baumannii is one of the most problematic nosocomial pathogens that can efficiently thrive within hospital settings, mainly due to resistances toward antibiotics, desiccation, disinfectants, human serum and oxidative stress. Recently, increased resistance against last-resort antibiotics earns this bacterium the highest priority concern classified by the Centers for Disease Control and Prevention and the World Health Organization. An obvious hallmark of this bacterium is the high heterogeneity observed among *A. baumannii* isolates, with a limited core genome. This feature complexifies the study of *A. baumannii* bacteria as an entity, subsequently reflected in a diversity of phenotypes of not only antimicrobial and environmental resistance but also virulence. A high degree of genome plasticity, along with the use of a limited subset of established strains, can lead to strain-specific observations, decreasing the global understanding of this pathogenic agent. Phenotypic variability of *A. baumannii* strains is easily observable such as with the macrocolony morphologies, *in vitro* and *in vivo* virulence, natural competence level, production of different capsular polysaccharide structures and cellular densities. Some strains encode several virulence factors, while others, including the established strains, lack key ones. The lack/excess of genes or specific physiological processes might interfere with *in vivo* and *in vitro* experiments, thus providing a limited impact on the global understanding of *Acinetobacter* bacteria. As an answer to the high heterogeneity among *A. baumannii* strains, we propose a first comprehensive database that includes the bacterial strains and the associated phenotypic and genetic data. This new repository, freely accessible to the entire scientific community, allows selecting the best bacterial isolate(s) related to any biological question, using an efficient and fast exchange platform.

Database URL: <https://acinetobase.vib.be/>

Introduction

Acinetobacter baumannii is an opportunistic nosocomial pathogen thriving in hospital environment and endangering, especially critically ill patients (1). This Gram-negative bacterium is part of the most problematic ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) group of human pathogens, against which therapeutic approaches become limited (2). Increased isolations of antibiotic-resistant strains, especially to last-resort antibiotics, earn this bacterium the consideration as an ‘urgent threat to public health’ by the Centers for Disease Control and Prevention (3) and as ‘a bacterium for which research and development of new antibiotics is critically needed’ by the World Health Organization (4). An obvious hallmark of *A. baumannii* bacteria is the high heterogeneity observed among the isolates (5). *Acinetobacter baumannii* bacteria have a dynamic genome, with an estimated conserved core genome of only 16.5%, while 25% of the genome is unique to each strain, meaning that there is no counterpart in any other

A. baumannii genome (5). Our recent study (6) on 47 strains of *A. baumannii* shows even lower core genome of 2009 genes from the pangenome of 13 611 genes, accounting for 14.76%. *Acinetobacter baumannii* isolates are impressively variable, at both genetic and phenotypic levels. For example, the capsular polysaccharide, which impacts antibiotic and environmental resistances, host response and virulence, is encoded by at least 237 various capsule locus types, while the outer core of lipooligosaccharide is encoded by at least 13 locus types (7, 8). This genetic variability translates into phenotypic heterogeneity, observable using electron microscopy where the thickness and rigidity of the capsule differ, along with different cellular densities (9). The established strains such as ATCC19606, ATCC17978, DSM30011 or AB5075 are widely used type strains from which significant and validated observations were and are still generated (10–13). Altogether, these established strains greatly contributed to the state of the art of the current and growing *A. baumannii* field. The established strains AB5075, ATCC17978 and ATCC19606 are multidrug-resistant (MDR) strains of clinical origin

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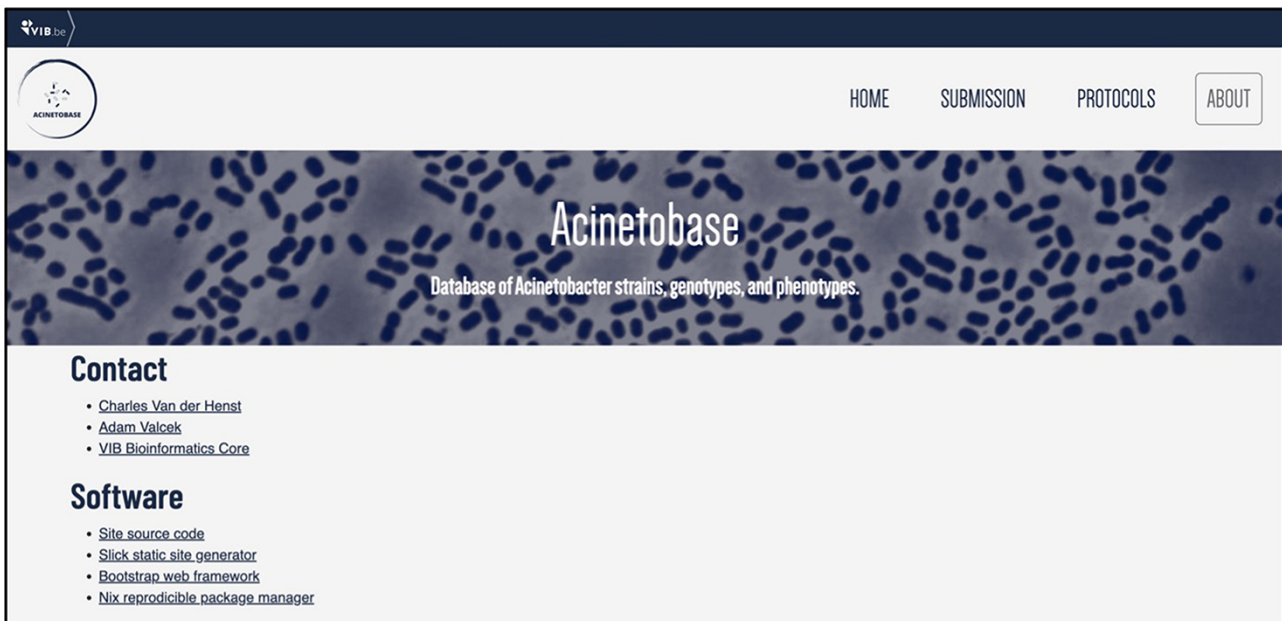


Figure 1. 'About' page of Acinetobase.

(14, 15), while DSM30011 is a non-MDR environmental strain obtained from plant microbiota (16). The observations gained about these established strains shed a strong base of the knowledge about *A. baumannii*. However, on the other hand, type strains also showed the limitations of only using these strains when current clinical isolates of *A. baumannii* show extreme genetic and phenotypic variabilities (6). In order to fill this modern gap of knowledge, grasp the complexity of *A. baumannii* and widen the possibilities of the community to use and efficiently share genotypically and phenotypically characterized strains, we propose the database and strain repository Acinetobase as a new community tool (Figure 1). This new data and strains sharing platform is especially important in the context of novel therapeutic discoveries, for which conserved targets and antimicrobial modes of actions among the highest proportion of problematic and clinically relevant *A. baumannii* isolates are required.

Results

Construction and content

This first version of Acinetobase consists of 43 clinical isolates, 4 widely used strains (AB5075-VUB, ATCC19606-VUB, ATCC17978-VUB and DSM30011-VUB) and 1 naturally occurring mutant of *A. baumannii* AB5075-VUB, disrupted in the *itrA* gene designated AB5075-VUB-*itrA*::*ISAb13* (9). We generated whole-genome sequences and *de novo* assembled the complete chromosomal sequences with high quality of all these strains, rendering them publicly available via GenBank accession number. In addition, the Acinetobase includes a variety of phenotypical characteristics associated with a strain:

- (i) Quantification of the capsular polysaccharide using the gradient density method for each strain (9)
- (ii) Type of macrocolony produced for each strain (designated with letters A to F) with a corresponding photograph (6)

- (iii) Virulence in *Galleria mellonella* infection model for nine strains (9)
- (iv) Transmission electron microscopy (TEM) photograph depicting the amount and density of the capsule for 11 strains (6)
- (v) Antimicrobial susceptibility testing with minimum inhibitory concentrations for 43 clinical isolates (20)

The database also describes the basic genotypical characteristics of each strain:

- (i) Multilocus sequence type (ST) according to Pasteur (17) and Oxford (18, 19) typing scheme
- (ii) Capsule locus (KL) type (7, 21)
- (iii) Outer core lipooligosaccharide locus type (OCL) (7, 21)
- (iv) Antibiotic resistance genes (ARGs) obtained using the ResFinder 4.0 (24) database as described earlier (20)

Furthermore, the metadata for each strain are provided along with a reference to the publication providing a more profound description:

- (i) Origin of the strain (e.g. clinical, environmental and animal)
- (ii) Source of isolation (e.g. wound, sputum and feces)
- (iii) Year of isolation

The above-mentioned genotypical and phenotypical traits along with metadata can be used to search the database and filter the strains for specific criteria. This way users can efficiently find the strain of desired characteristics and request it. The bacterial strains of *A. baumannii* were deposited and are available in lyophilized form as per order from the Belgian Coordinated Collection of Microorganisms/Lab of Microbiology (<https://bccm.belspo.be/>). The example of an entry of AB5075-VUB is depicted in Figure 2.

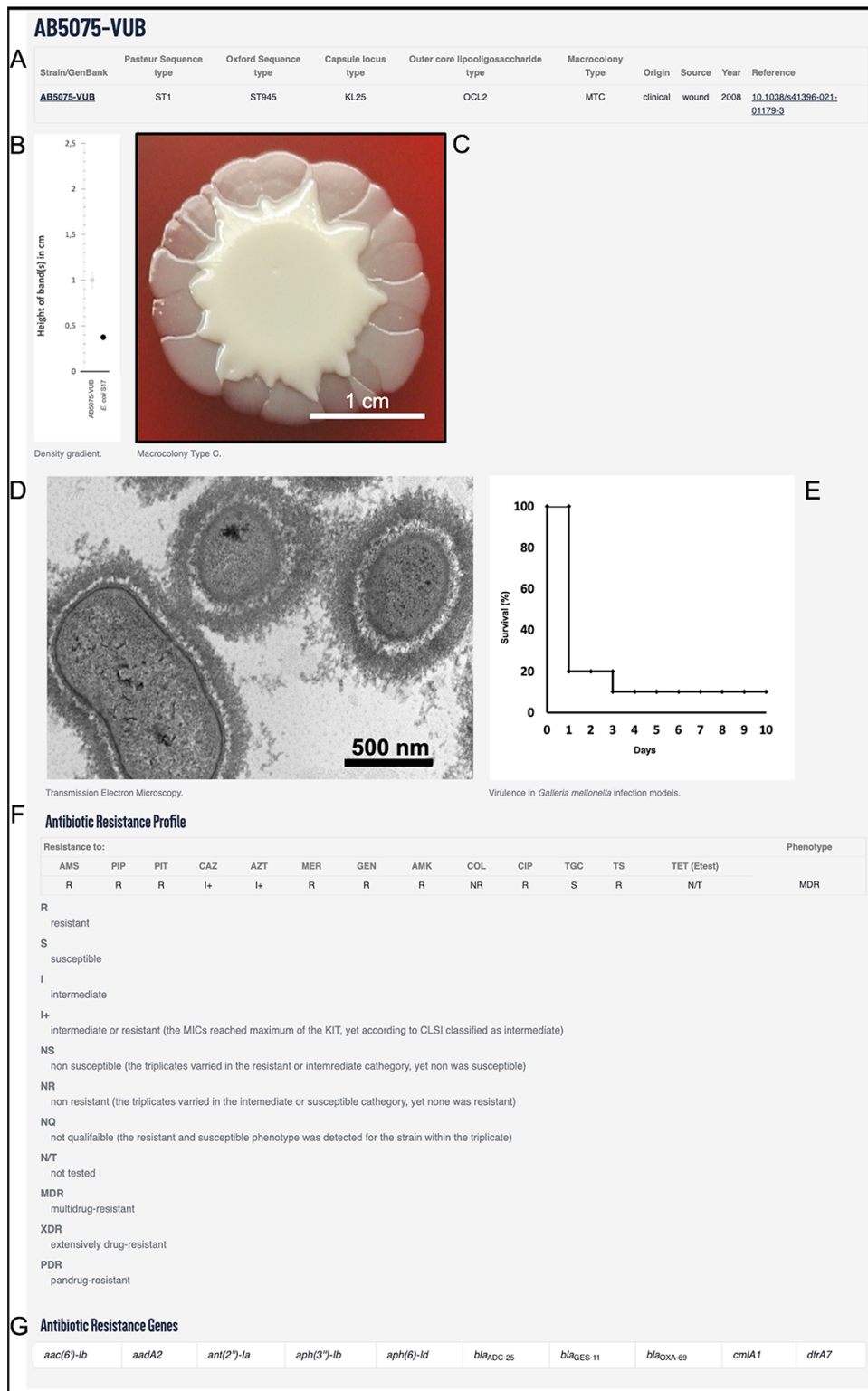


Figure 2. Example using AB5075-VUB entry depicting: (A) description of the genotype (KL, ST0x/Pas and OCL), origin, source and year of isolation with a hyperlink for the complete chromosomal sequence and a reference publication; (B) quantification of the capsule; (C) macrocolony type; (D) TEM micrograph of the capsule; (E) virulence in *G. mellonella* infection model; (F) antibiotic resistance profile and (G) ARGs.

Discussion

Existing databases

The decreasing costs of the whole-genome sequencing allowed genomic databases, filled with high-quality data, to thrive.

There are well-established databases such as EnteroBase (22), which do not include data on *Acinetobacter*. In contrast, existing databases that provide whole-genome sequencing data on *A. baumannii*, such as BIGSdb (23), include metadata

Strain	Pasteur Sequence type	Oxford Sequence type	Capsule locus type	Outer core lipooligosaccharide type	Macrocolony Type	Source
AB14-VUB	ST636	ST502	KL40	OCL2	MTC	wound
AB16-VUB	ST2	ST218	KL7	OCL1	MTB	endotracheal aspirates
AB167-VUB	ST2	ST451	KL6	OCL1	MTD	endotracheal aspirates
AB169-VUB	ST1	ST405	KL18	OCL1	MTC	throat

Figure 3. ‘Collection’ page depicting the name, Pasteur and Oxford STs, KL, OCL, macrocolony types and source of each isolate.

and genotype; however, BIGSdb does not include phenotypic data unrelated to the genotype nor the strains themselves. Moreover, centralization of the laboratory providing strains with the genotype and phenotype characteristics will help to decrease the chance of working with subcultured variants with altered genotype and phenotype, a pitfall that becomes more and more apparent in the *A. baumannii* field (9, 25, 26).

Browsing

The users can browse the ‘Collection’ page for the strains (Figure 3) and pick according to their genomic description. By clicking on the name of the selected strain, the full entry with genomic and phenotypic details appears (Figure 2).

Searching

The users can filter the entries (Figure 4) according to each genetic- and phenotypic-based criterion depicted in the ‘Collection’ page. This feature helps to select specific strains of *A. baumannii* according to criteria demanded by the user, appropriate to their biological question.

Download

To avoid unnecessary redundancy, downloading the complete chromosomal sequences and the whole-genome assemblies is facilitated via GenBank (<https://www.ncbi.nlm.nih.gov/>). The source information on the strains can be found in the corresponding research articles found in the ‘Reference’ column using hyperlinks (using a digital object identifier). However, the main information such as metadata for each strain and protocols for each methodology is displayed in Acinetobase. In the corresponding peer-reviewed study, authors of the study provide additional or further information on how the strains were obtained their origin, original figures and the protocols for assessing the phenotypical traits. The authors of the study will be directly responsible for the information and data they provided and submitted to Acinetobase.

Submissions

The Acinetobase is open for submissions of the genomic and phenotypic data of *Acinetobacter* strains by other research teams and laboratories. However, there are requirements that must be met prior to contacting the curators of the Acinetobase with an inquiry of submission. We also propose ‘Optional’ information to be provided by submitters. The requirements and optional information are discussed in the next subsection.

Requirements

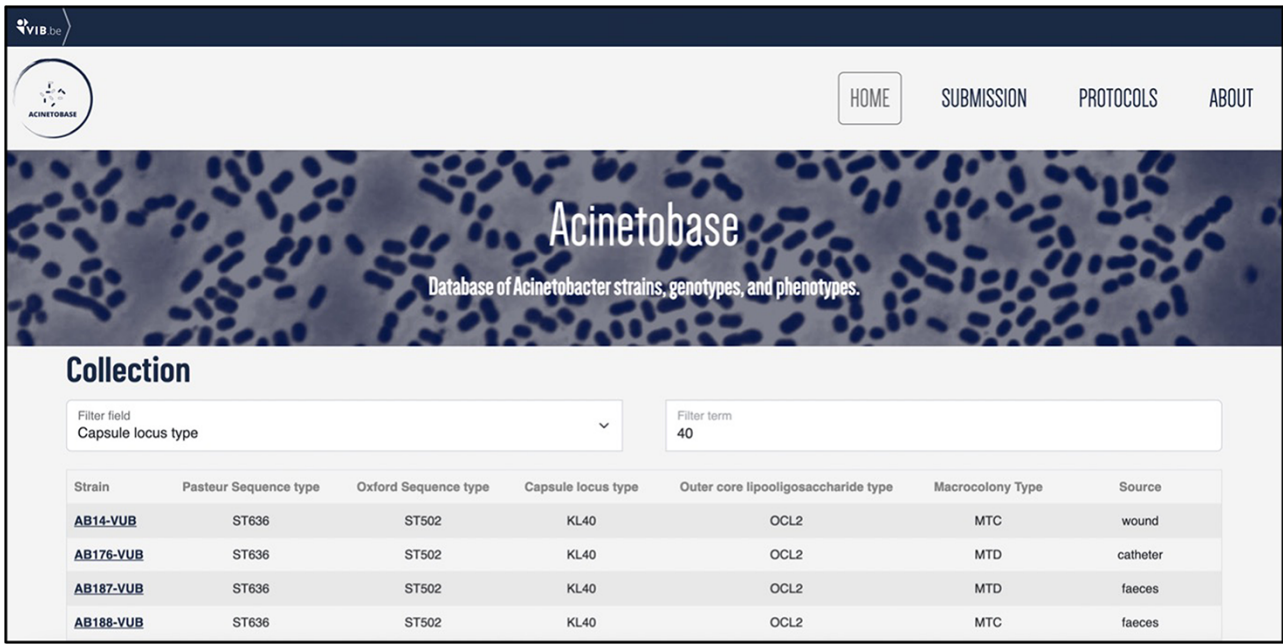
- (i) You are willing to share the strain upon demand.
- (ii) You can provide information on the strain such as the source of isolation and year (no Protected Health Information).

Optional

- (i) The maximum genetic and phenotypic characterization.
- (ii) The hyperlink for the whole-genome sequences within a repository (e.g. GenBank)—optimally complete genomes (closed circular genomes).
- (iii) Reference

Future perspectives

Acinetobase has a significant potential to influence the field of microbiology, especially the *A. baumannii* community, and other research fields working with similar pathogens and biological questions might be inspired. Further implementation potential of Acinetobase resides in new strain acquisition and the accumulation of additional experimental analyses and data. This will provide valuable information to researchers on whether one (or a subset of available strains) is an appropriate isolate to work with, depending on the biological question aimed. The proposed project is so far aimed to *A. baumannii*, yet not limited to a single species of *Acinetobacter*. With an increasing clinical relevance of other *Acinetobacter*



The screenshot shows the Acinetobase website interface. At the top, there is a navigation bar with links for HOME, SUBMISSION, PROTOCOLS, and ABOUT. Below this is a banner with the Acinetobase logo and the text 'Database of Acinetobacter strains, genotypes, and phenotypes.' The main content area is titled 'Collection' and features a search filter for 'Capsule locus type' with a dropdown menu and a search term '40'. Below the filter is a table with the following data:

Strain	Pasteur Sequence type	Oxford Sequence type	Capsule locus type	Outer core lipooligosaccharide type	Macrocolony Type	Source
AB14-VUB	ST636	ST502	KL40	OCL2	MTC	wound
AB176-VUB	ST636	ST502	KL40	OCL2	MTD	catheter
AB187-VUB	ST636	ST502	KL40	OCL2	MTD	faeces
AB188-VUB	ST636	ST502	KL40	OCL2	MTC	faeces

Figure 4. Example of entries filtering based on the KL type 40.

species such as *A. pittii*, *A. nosocomialis*, *A. seifertii* and *A. dijkshoorniae* (27), the Acinetobase initiative is open to all *Acinetobacter* species. Acinetobase accepts submission of the phenotypic and genotypic data including strains from other laboratories and research groups, and the authors (curators) of the Acinetobase do intend to provide further genomic and phenotypic data in form of strain submissions. This initiative will, over time, result in a growing database and a platform for sharing knowledge and *Acinetobacter* spp. strains and might include further molecular tools such as fluorescently labeled strains or characterized deletion mutant strains.

Conclusion

In conclusion, Acinetobase is the first database dedicated to *Acinetobacter* genotypes and associated phenotypes that also serves as a repository of characterized strains. Acinetobase not only will be the proof of concept of high heterogeneity among *A. baumannii* strains but also will transform this pitfall into a new strength, benefiting the entire community by helping to select an appropriate strain related to specific conditions, experiments and biological questions. The subculturing steps of the current strain-sharing strategy will therefore decrease, helping to keep the genetic background of these dynamic bacteria as much as possible.

Data availability

The datasets generated and analyzed during the current study are linked from the Acinetobase website (<http://acinetobase.vib.be/>).

Author contributions

A.V. and C.V.d.H. wrote the manuscript. C.V.d.H. conceptualized the Acinetobase. J.C. wrote and designed the website,

data storage and update mechanisms. A.B. assisted with the design of the website, is the project sponsor and reviewed the manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare that they have no competing interests.

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