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

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Abstract

Background

Acinetobacter baumannii is one of the most problematic nosocomial pathogens that can efficiently thrive within hospital settings, mainly due to resistances towards antibiotics, desiccation, disinfectants, human serum and oxidative stress. Recently, increased resistance against last resort antibiotics earns this bacterium a highest priority concern classified by the CDC and the WHO. An obvious hallmark of this bacterium is the high heterogeneity observed amongst *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.

Main body

High degree of genome plasticity, along with the use of a limited subset of established reference 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 microcolony morphologies, *in vitro* and *in vivo* virulence, production of different capsular polysaccharide structures and cellular densities. Some strains encode an extensive amount of virulence factors while other, including the reference strains, lack several key ones. The lack/excess of genes or specific physiological processes might interfere with *in vivo* and *in vitro* experiments, thus providing limited impact on the global understanding of *Acinetobacter* bacteria.

Conclusion

As an answer to the high heterogeneity amongst *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 community, allow selecting the best bacterial isolate(s) related to any biological question, using an efficient and fast exchange platform.

Keywords

Acinetobase, *Acinetobacter baumannii*, phenotypes, genotypes, database, repository

Background

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 group of human pathogens, against which therapeutic approaches become limited [2]. Increased isolations of antibiotic-resistant strains, especially to last resort antibiotics, earns this bacterium the consideration as "urgent threat to public health" by the Centers for Disease Control and Prevention (CDC) [3] and as "a bacterium for which research and development of new antibiotics is critically needed" by the World Health Organization (WHO) [4]. An obvious hallmark of *A. baumannii* bacteria is the high heterogeneity observed amongst the isolates [5]. *A. 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 (Philippe et al., 2022, unpublished) on 47 strains of *A. baumannii* shows even lower core genome of 2009 genes from the pangenome of 13611 genes, accounting for 14.76%. *A. 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 137 various capsule locus types, while the outer core of lipooligosaccharide by at least 13 locus types [6, 7]. This genetic variability translates into phenotypic heterogeneity, observable using electron microscopy where the thickness and rigidity of the capsule differs, along with different cellular densities [8]. The established reference strains such as ATCC19606, ATCC17978, DSM30011 or AB5075 are widely used type strains from which significant and validated observations were and are still generated [9–12]. All together, these reference strains greatly contributed to the state of the art of the current and growing *A. baumannii* field. The reference strains AB5075, ATCC17978 and ATCC19606 are multidrug resistant (MDR) strains of clinical origin [17, 18]

while DSM30011 is a non-MDR environmental strain obtained from plant microbiota [19]. The observations gained about these reference strains shed a strong base of the knowledge about *A. baumannii*. However, in 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. 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 (Fig. 1). This new data and strains sharing platform is especially important in the context of novel therapeutic discoveries, for which conserved targets and antimicrobial mode of actions amongst the highest proportion of problematic and clinically relevant *A. baumannii* isolates is required.

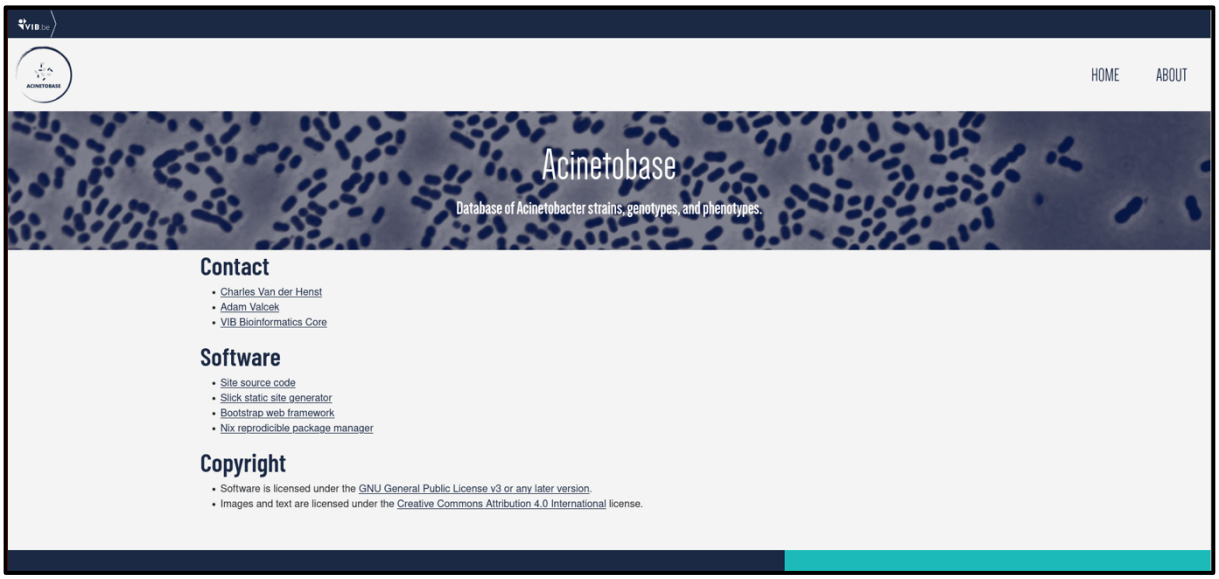


Figure 1: The view of the "About" page of Acinetobase.

Construction and content

This first version of Acinetobase consists of 43 clinical isolates and four sub-cultured reference strains (AB5075-VUB, ATCC19606-VUB, ATCC17978-VUB and DSM30011-VUB) of *A. baumannii*. All these strains where whole genome sequenced and the complete chromosomal

88 sequences of high quality are publicly available via GenBank accession number, where they
89 were deposited. In addition, the Acinetobase includes a variety of phenotypical characteristics
90 associated with a strain:

91 i, Quantification of the capsular polysaccharide using gradient density method for each
92 strain [12]

93 ii, Type of macrocolony produced for each strain (designated with letter A to E) with a
94 corresponding photograph

95 iii, Virulence in *Galleria mellonella* infection model for 9 strains [12]

96 iv, Transmission electron microscopy (TEM) photograph depicting the amount and
97 density of the capsule for 11 strains

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

99 i, Multilocus sequence type (ST) according to Pasteur [20] and Oxford [21, 22] typing
100 scheme

101 ii, Capsule locus type (KL) [6, 19]

102 iii, Outer core lipooligosaccharide locus type (OCL) [6, 19]

103 The above mentioned genotypical and phenotypical traits can be used to search the database
104 and filter the strains for specific criteria. This way users can efficiently find the strain of desired
105 characteristics and request it.

106 The bacterial strains of *A. baumannii* were deposited and are available in lyophilized form as
107 per order from the Belgian Coordinated Collection of Microorganisms/Lab of Microbiology
108 (BCCM/LMG) (https://bccm.belspo.be/).

109 The example of an entry of AB5075-VUB is depicted in Fig. 2.

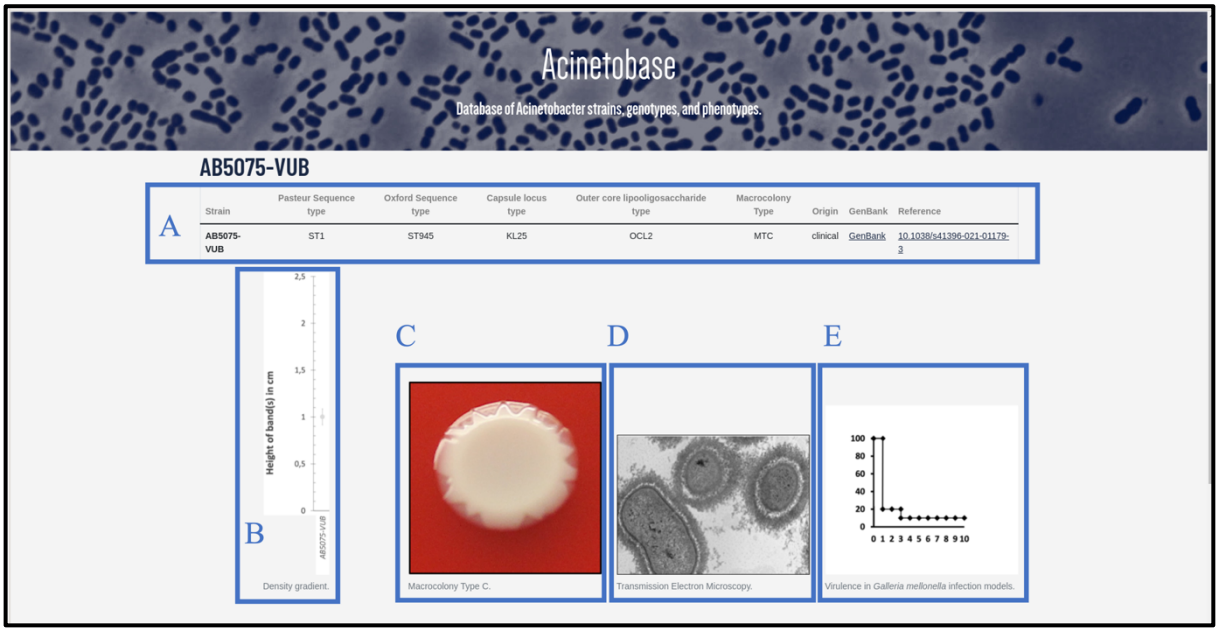


Figure 2: An entry of AB5075-VUB depicting A, description of the genotype (KL, $ST^{Ox/Pas}$, OCL) with a hyperlink for the complete chromosomal sequence and a reference publication; B, quantification of the capsule; C, macrocolony type; D, TEM micrograph of the capsule and E, virulence in *Galleria mellonella* infection model.

Utility and 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 [20], which does not include data on *Acinetobacter*. In contrast, existing databases which provide whole-genome sequencing data on *A. baumannii*, such as BIGSdb [21], includes metadata 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 variant which might have altered genotype and phenotype of the parental strain (Valcek et al., unpublished).

127 **Browsing**

128 The users can browse the "Collection" page for the strains (Fig. 3) and pick according to their
129 genomic description. By clicking on the name of the selected strain the full entry with genomic
130 and phenotypic details appears (Fig. 2).

131

132 **Searching**

133 The users can filter the entries (Fig. 4) according to each genetic and phenotypic-based
134 criterium depicted in the "Collection" page. This feature helps to select specific strains of *A.*
135 *baumannii* according to criteria demanded by the user, appropriated to their biological
136 question.

137

138 **Download**

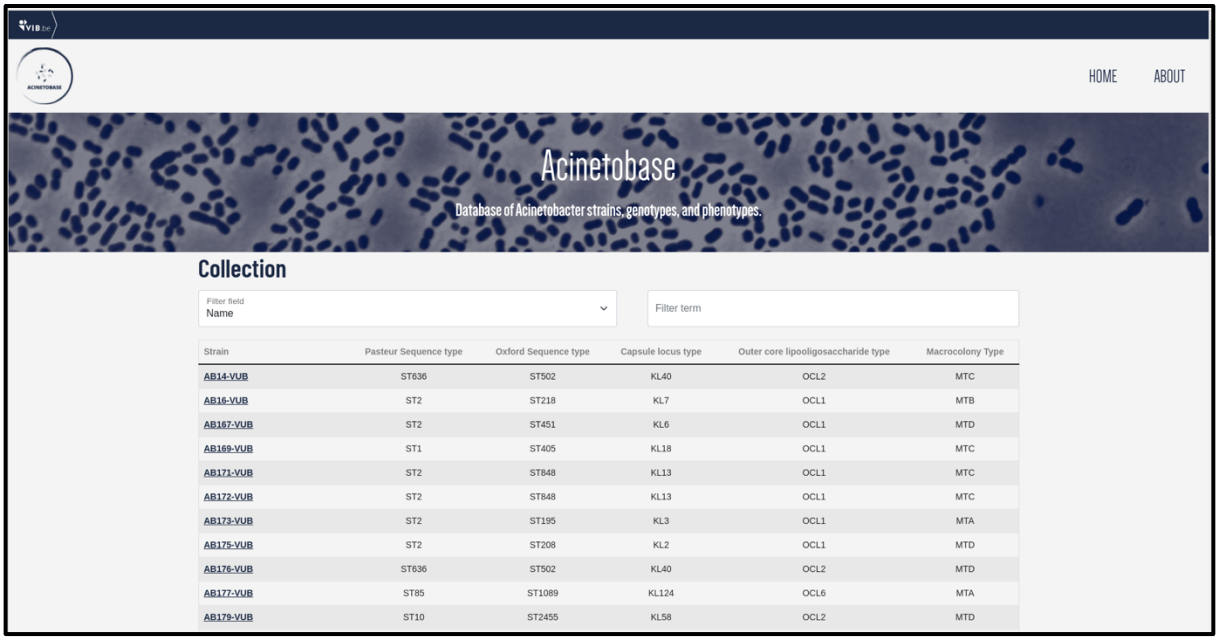
139 To avoid unnecessary redundancy, downloading the complete chromosomal sequences and the
140 whole-genome assemblies is facilitated via GenBank (https://www.ncbi.nlm.nih.gov/). The
141 source information on the strains can be found in the corresponding research articles found in
142 the "Reference" column. By hyperlinking (using Digital Object Identifier - DOI) the
143 corresponding peer-reviewed study, authors of the study provide the protocol on how the
144 strains were obtained, their origin, original figures, and the protocols for assessing the
145 phenotypical traits. The authors of the study will be directly responsible for the information
146 and data the provided and submitted to Acinetobase.

147

148 **Future perspectives**

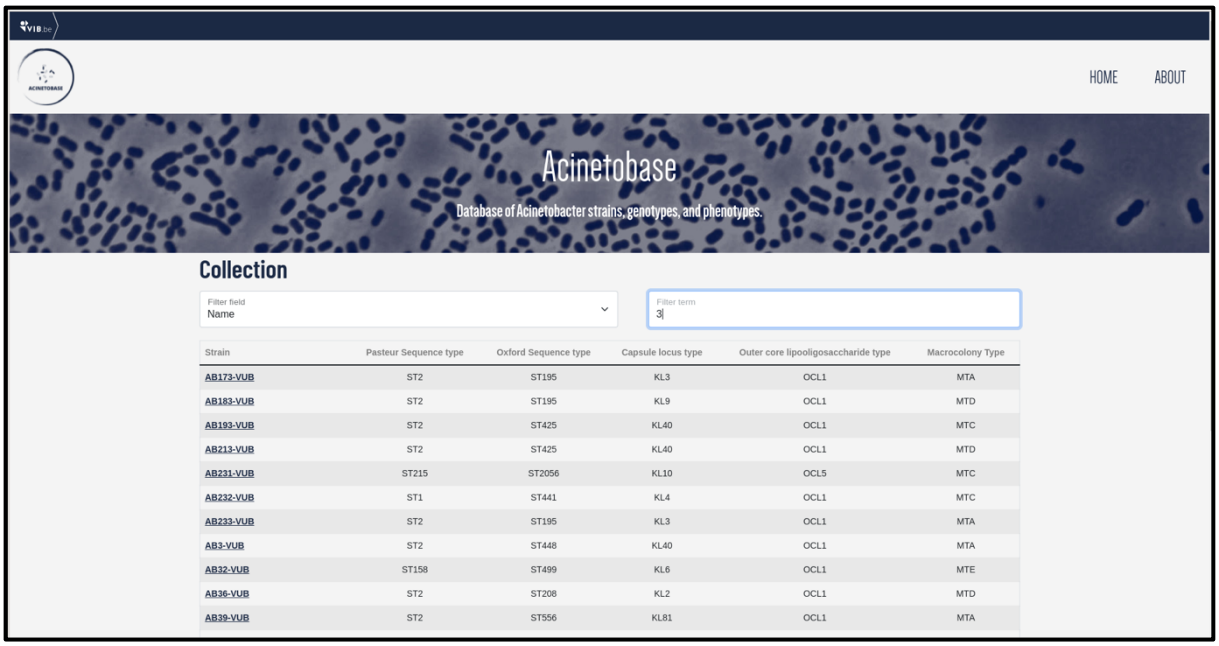
149 Acinetobase has a great potential to influence the field of microbiology, especially the *A.*
150 *baumannii* community, but also other communities which work with similar pathogens (such
151 as *Klebsiella pneumoniae*) and similar biological questions, might be inspired. Further

implementation potential of Acinetobase resides in additional experimental analyses which can be easily and quickly performed and provide valuable information to researchers whether it is a correct strain to work with (e.g., if the isolate is capable of natural transformation or if it forms biofilm). The proposed project is so far aimed to *Acinetobacter baumannii*, yet not limited to a single species of *Acinetobacter*. With an increasing clinical relevance of other *Acinetobacter* species such as *A. pittii*, *A. nosocomialis*, *A. seifertti* and *A. dijkshoorniae* [22], the project of Acinetobase is open to all *Acinetobacter* species. Acinetobase will be open to submission of the phenotypic and genotypic data including strains from other laboratories and research groups. 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.



Strain	Pasteur Sequence type	Oxford Sequence type	Capsule locus type	Outer core lipooligosaccharide type	Macrocolony Type
AB14-VUB	ST636	ST502	KL40	OCL2	MTC
AB16-VUB	ST2	ST218	KL7	OCL1	MTB
AB167-VUB	ST2	ST451	KL6	OCL1	MTD
AB169-VUB	ST1	ST405	KL18	OCL1	MTC
AB171-VUB	ST2	ST848	KL13	OCL1	MTC
AB172-VUB	ST2	ST848	KL13	OCL1	MTC
AB173-VUB	ST2	ST195	KL3	OCL1	MTA
AB175-VUB	ST2	ST208	KL2	OCL1	MTD
AB176-VUB	ST636	ST502	KL40	OCL2	MTD
AB177-VUB	ST85	ST1089	KL124	OCL6	MTA
AB179-VUB	ST10	ST2455	KL58	OCL2	MTD

Figure 3: The "Collection" page depicting the name, Pasteur and Oxford sequence type, KL, OCL and macrocolony types of each isolate in the collection.



Strain	Pasteur Sequence type	Oxford Sequence type	Capsule locus type	Outer core lipooligosaccharide type	Macrocolony Type
AB173-VUB	ST2	ST195	KL3	OCL1	MTA
AB183-VUB	ST2	ST195	KL9	OCL1	MTD
AB193-VUB	ST2	ST425	KL40	OCL1	MTC
AB213-VUB	ST2	ST425	KL40	OCL1	MTD
AB231-VUB	ST215	ST2056	KL10	OCL5	MTC
AB232-VUB	ST1	ST441	KL4	OCL1	MTC
AB233-VUB	ST2	ST195	KL3	OCL1	MTA
AB3-VUB	ST2	ST448	KL40	OCL1	MTA
AB32-VUB	ST158	ST499	KL6	OCL1	MTE
AB36-VUB	ST2	ST208	KL2	OCL1	MTD
AB39-VUB	ST2	ST556	KL81	OCL1	MTA

Figure 4: An example of filtering the entries based on the number 3 in the name of the isolate.

Conclusion

In conclusion, Acinetobase is the first database dedicated to *Acinetobacter* genotypes and associated phenotypes that also serves as a repository of strain. Acinetobase will not only be the proof-of-concept of high heterogeneity among *A. baumannii* strains but will also benefit the entire community by helping to select an appropriate strain related to specific conditions, experiments, and biological questions.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AV and CVDH wrote up the manuscript. CVDH conceptualized the Acinetobase. JC wrote and designed the website, data storage, and update mechanisms. AB assisted with design of the website, is the project sponsor and reviewed the manuscript.

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