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

Adam Valcek[1, 2], James Collier[3], Alexander Botzki[3] and Charles Van der Henst[1, 2,#]

[#] Corresponding author: charles.vanderhenst@vub.vib.be

Affiliations

[1] Microbial Resistance and Drug Discovery, VIB-VUB Center for Structural Biology, VIB, Flanders Institute for Biotechnology, Brussels, Belgium.

[2] Structural Biology Brussels, Vrije Universiteit Brussel (VUB), Brussels, Belgium.

[3] VIB Bioinformatics Core, Ghent, Belgium

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 the 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 macrocolony morphologies, *in vitro* and *in vivo* virulence, natural competence level, 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 as a whole.

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 scientific community, allow selecting the best bacterial isolate(s) related to any biological question, using an efficient and fast exchange platform.

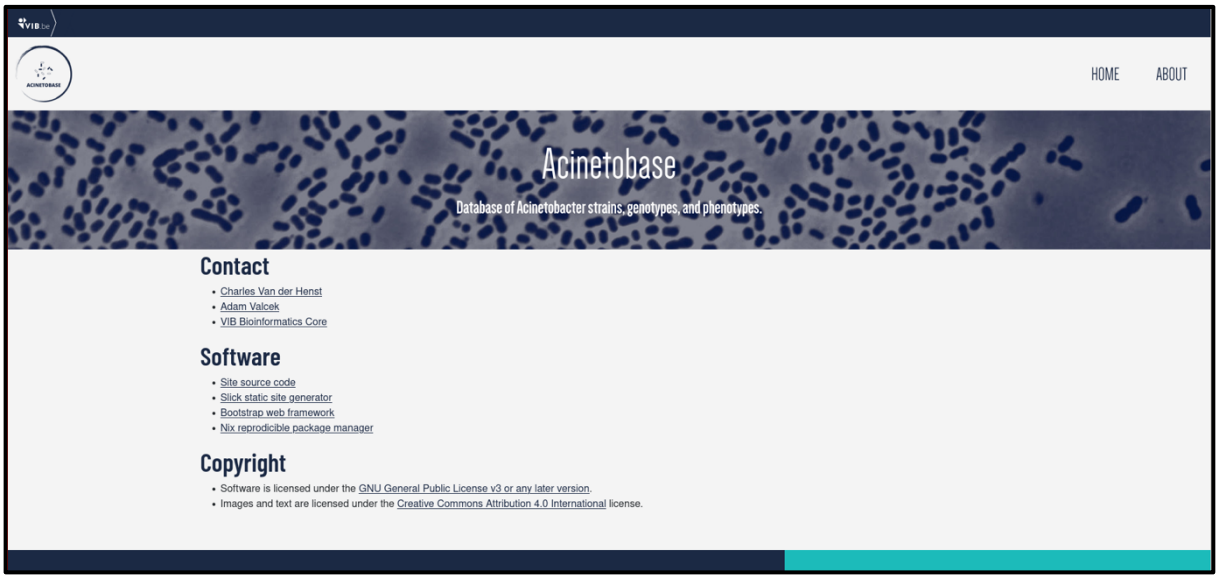
Keywords

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

46 **Background**

47 *Acinetobacter baumannii* is an opportunistic nosocomial pathogen thriving in hospital
48 environment and endangering especially critically ill patients (Whiteway *et al.*, 2022). This
49 Gram-negative bacterium is part of the most problematic ESKAPE group of human pathogens,
50 against which therapeutic approaches become limited (Pendleton *et al.*, 2014). Increased
51 isolations of antibiotic-resistant strains, especially to last resort antibiotics, earns this bacterium
52 the consideration as "urgent threat to public health" by the Centers for Disease Control and
53 Prevention (CDC) (CDC. Antibiotic Resistance Threats in the United States and U.S.
54 Department of Health and Human Services) and as "a bacterium for which research and
55 development of new antibiotics is critically needed" by the World Health Organization (WHO)
56 (Tacconelli *et al.*, 2018). An obvious hallmark of *A. baumannii* bacteria is the high
57 heterogeneity observed amongst the isolates [5]. *A. baumannii* bacteria have a dynamic
58 genome, with an estimated conserved core genome of only 16.5%, while 25% of the genome
59 is unique to each strain, meaning that there is no counterpart in any other *A. baumannii* genome
60 (Imperi *et al.*, 2011). Our recent study (Philippe *et al.*, 2022) on 47 strains of *A. baumannii*
61 shows even lower core genome of 2009 genes from the pangenome of 13611 genes, accounting
62 for 14.76%. *A. baumannii* isolates are impressively variable, at both genetic and phenotypic
63 levels. For example, the capsular polysaccharide, which impacts antibiotic and environmental
64 resistances, host response and virulence, is encoded by at least 137 various capsule locus types,
65 while the outer core of lipooligosaccharide by at least 13 locus types (Wyres *et al.*, 2020;
66 Kenyon and Hall, 2021). This genetic variability translates into phenotypic heterogeneity,
67 observable using electron microscopy where the thickness and rigidity of the capsule differs,
68 along with different cellular densities (Whiteway *et al.*, 2021). The established reference strains
69 such as ATCC19606, ATCC17978, DSM30011 or AB5075 are widely used type strains from
70 which significant and validated observations were and are still generated (Roussin *et al.*, 2019;

71 Bravo *et al.*, 2016; Jacobs *et al.*, 2014; de Silva *et al.*, 2017). All together, these reference
72 strains greatly contributed to the state of the art of the current and growing *A. baumannii* field.
73 The reference strains AB5075, ATCC17978 and ATCC19606 are multidrug resistant (MDR)
74 strains of clinical origin [17, 18] while DSM30011 is a non-MDR environmental strain
75 obtained from plant microbiota [19]. The observations gained about these reference strains
76 shed a strong base of the knowledge about *A. baumannii*. However, in the other hand, type
77 strains also showed the limitations of only using these strains when current clinical isolates of
78 *A. baumannii* show extreme genetic and phenotypic variabilities (Philippe *et al.*, 2022). In order
79 to fill this modern gap of knowledge, grasp the complexity of *A. baumannii* and widen the
80 possibilities of the community to use and efficiently share genotypically and phenotypically
81 characterized strains, we propose the database and strain repository Acinetobase as a new
82 community tool (Fig. 1). This new data and strains sharing platform is especially important in
83 the context of novel therapeutic discoveries, for which conserved targets and antimicrobial
84 mode of actions amongst the highest proportion of problematic and clinically relevant *A.*
85 *baumannii* isolates is required.

86 

87 **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 reference strains (AB5075-VUB, ATCC19606-VUB, ATCC17978-VUB and DSM30011-VUB) of *A. baumannii*. 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 gradient density method for each strain [12]

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

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

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

The database also describes basic genotypical characteristics of each strain:

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

ii, Capsule locus type (KL) (Wick *et al.*, 2018; Wyres *et al.*, 2020)

iii, Outer core lipooligosaccharide locus type (OCL) (Wick *et al.*, 2018; Wyres *et al.*, 2020)

The above mentioned genotypical and phenotypical traits 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 (BCCM/LMG) (https://bccm.belspo.be/). The example of an entry of AB5075-VUB is depicted in Fig. 2.

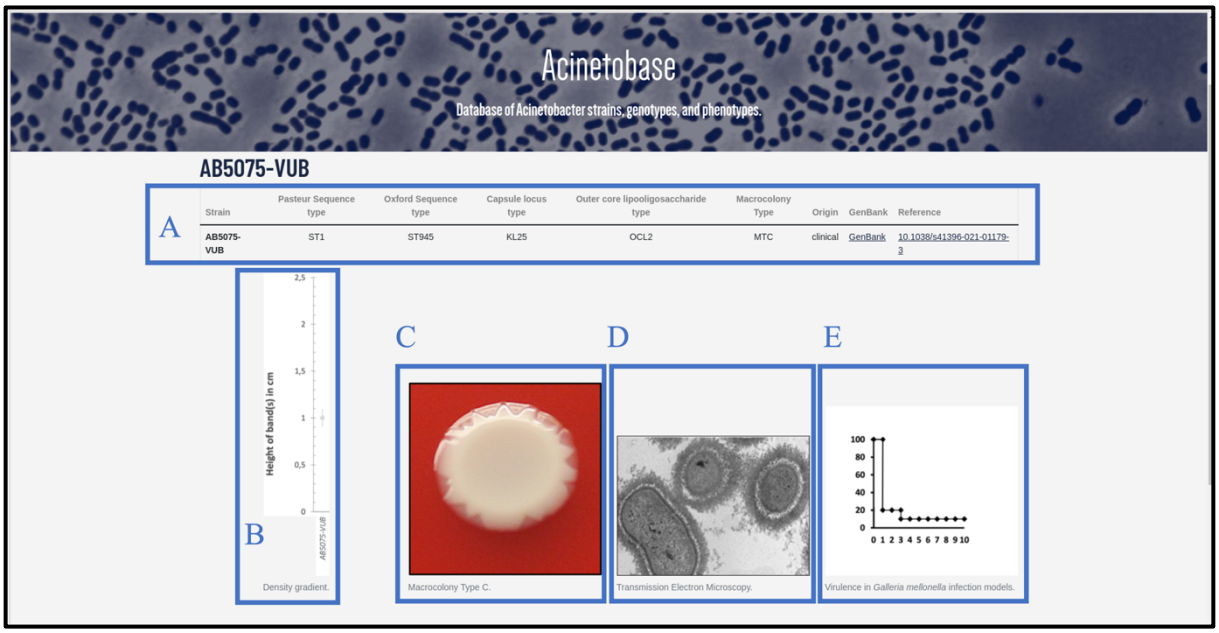


Figure 2: An entry of AB5075-VUB depicting **A**, description of the genotype (KL, $ST^{Ox/Pas}$, OCL) with a hyperlink for the complete chromosomal sequence and a reference publication; **B**, quantification of the capsule; **C**, macrocolony type; **D**, TEM micrograph of the capsule and **E**, virulence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

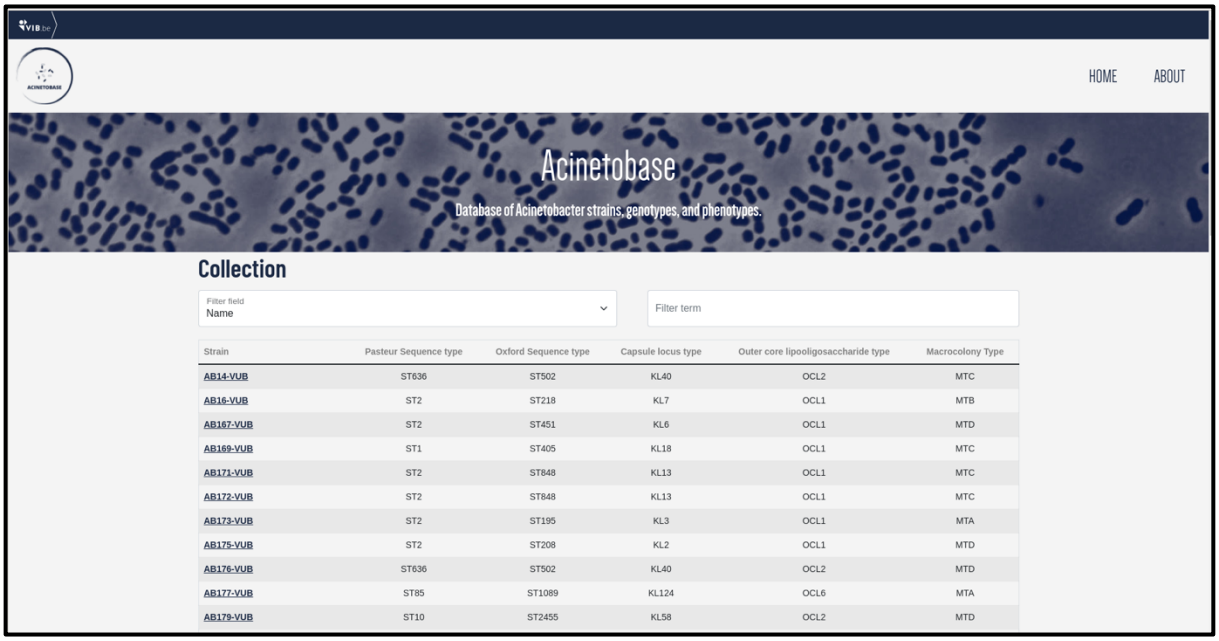
Utility and discussion

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 (Zhou *et al.*, 2020), which does not include data on *Acinetobacter*. In contrast, existing databases which provide whole-genome sequencing data on *A. baumannii*, such as BIGSdb (Jolley *et al.*, 2018), 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 variants with altered genotype and phenotype, a pitfall that becomes

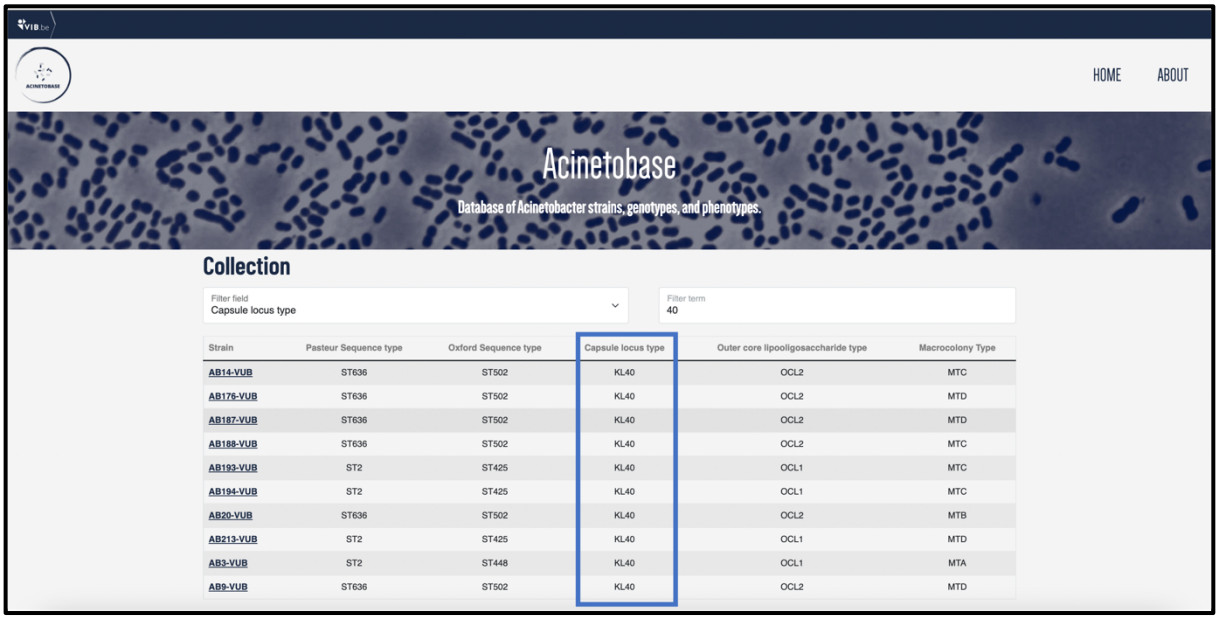
129 more and more apparent in the *A. baumannii* field (Whiteway *et al.*, 2021; Artuso *et al.*, 2022;
130 Wijers *et al.*, 2021).
131
132 **Browsing**
133 The users can browse the "Collection" page for the strains (Fig. 3) and pick according to their
134 genomic description. By clicking on the name of the selected strain the full entry with genomic
135 and phenotypic details appears (Fig. 2).
136
137 **Searching**
138 The users can filter the entries (Fig. 4) according to each genetic and phenotypic-based
139 criterium depicted in the "Collection" page. This feature helps to select specific strains of *A.*
140 *baumannii* according to criteria demanded by the user, appropriated to their biological
141 question.
142
143 **Download**
144 To avoid unnecessary redundancy, downloading the complete chromosomal sequences and the
145 whole-genome assemblies is facilitated via GenBank (https://www.ncbi.nlm.nih.gov/). The
146 source information on the strains can be found in the corresponding research articles found in
147 the "Reference" column using hyperlinks (using Digital Object Identifier - DOI). In the
148 corresponding peer-reviewed study, authors of the study provide the protocol on how the
149 strains were obtained, their origin, original figures, and the protocols for assessing the
150 phenotypical traits. The authors of the study will be directly responsible for the information
151 and data they provided and submitted to Acinetobase.
152
153 **Future perspectives**

Acinetobase has a great potential to influence the field of microbiology, especially the *A. baumannii* community, but also other communities which work with similar pathogens and similar biological questions, might be inspired. Further implementation potential of Acinetobase resides in new strains acquisition and the accumulation of additional experimental analyses and data. This will provide valuable information to researchers whether one (or a subset of available strains) is an appropriate isolate to work with, depending of the biological question aimed. 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* (Vijayakumar *et al.*, 2019), 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

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



Strain	Pasteur Sequence type	Oxford Sequence type	Capsule locus type	Outer core lipooligosaccharide type	Macrocolony Type
AB14-VUB	ST636	ST502	KL40	OCL2	MTC
AB176-VUB	ST636	ST502	KL40	OCL2	MTD
AB187-VUB	ST636	ST502	KL40	OCL2	MTD
AB188-VUB	ST636	ST502	KL40	OCL2	MTC
AB193-VUB	ST2	ST425	KL40	OCL1	MTC
AB194-VUB	ST2	ST425	KL40	OCL1	MTC
AB20-VUB	ST636	ST502	KL40	OCL2	MTB
AB213-VUB	ST2	ST425	KL40	OCL1	MTD
AB3-VUB	ST2	ST448	KL40	OCL1	MTA
AB9-VUB	ST636	ST502	KL40	OCL2	MTD

173

174 **Figure 4:** An example of filtering the entries based on the Capsular locus (KL) type 40 (in the frame).

175 Conclusion

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

183

184

185
186

187

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.

Funding

This project was supported by the Flanders Institute for Biotechnology (VIB).

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.

Acknowledgements

We would like to thank to Belgian Coordinated Collections of Microorganisms of Ghent University, Faculty of Sciences, Belgium and the VIB Bioinformatics Core for their support in

developing the website, especially to Christof De Bo who helped with graphics. We also would like to thank the National Reference Laboratory for Monitoring of Antimicrobial Resistance in Gram-negative Bacteria, CHU Mont-Godinne, Université Catholique de Louvain (UCL), Yvoir, Belgium for sharing the modern clinical isolates of *A. baumannii*.

References

Artuso,I. *et al.* (2022) Genome diversity of domesticated *Acinetobacter baumannii* ATCC 19606T strains. *Microbial Genomics*, **8**, 000749.

Bartual,S.G. *et al.* (2005) Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. *Journal of Clinical Microbiology*, **43**, 4382–4390.

Bravo,Z. *et al.* (2016) The long-term survival of *Acinetobacter baumannii* ATCC 19606T under nutrient-deprived conditions does not require the entry into the viable but non-culturable state. *Archives of Microbiology*, **198**, 399–407.

CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta and U.S. Department of Health and Human Services. 2019. Antibiotic Resistance Threats in the United States, 2019.

Diancourt,L. *et al.* (2010) The Population Structure of *Acinetobacter baumannii*: Expanding Multiresistant Clones from an Ancestral Susceptible Genetic Pool. *PLOS ONE*, **5**, e10034.

Gallagher,L.A. *et al.* (2015) Resources for genetic and genomic analysis of emerging pathogen *Acinetobacter baumannii*. *Journal of Bacteriology*, **197**, 2027–2035.

Harding,C.M. *et al.* (2017) Uncovering the mechanisms of *Acinetobacter baumannii* virulence. *Nature Reviews Microbiology 2017 16:2*, **16**, 91–102.

Imperi,F. *et al.* (2011) The genomics of *Acinetobacter baumannii*: Insights into genome plasticity, antimicrobial resistance and pathogenicity. *IUBMB Life*, **63**, 1068–1074.

Jacobs,A.C. *et al.* (2014) AB5075, a highly virulent isolate of *Acinetobacter baumannii*, as a model strain for the evaluation of pathogenesis and antimicrobial treatments. *mBio*, **5**.

Jolley,K.A. *et al.* (2018) Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Research*, **3**.

Kenyon,J.J. and Hall,R.M. (2021) Updated Analysis of the Surface Carbohydrate Gene Clusters in a Diverse Panel of *Acinetobacter baumannii* Isolates. *Antimicrobial Agents and Chemotherapy*, **66**.

Pendleton,J.N. *et al.* (2014) Clinical relevance of the ESKAPE pathogens. *https://doi.org/10.1586/eri.13.12*, **11**, 297–308.

Philippe,C. *et al.* (2022) What are the reference strains of *Acinetobacter baumannii* referring to? *bioRxiv*, 2022.02.27.482139.

Repizo,G.D. *et al.* (2017) The Environmental *Acinetobacter baumannii* Isolate DSM30011 Reveals Clues into the Preantibiotic Era Genome Diversity, Virulence Potential, and Niche Range of a Predominant Nosocomial Pathogen. *Genome Biology and Evolution*, **9**, 2292–2307.

Roussin,M. *et al.* (2019) Identification of a Contact-Dependent Growth Inhibition (CDI) System That Reduces Biofilm Formation and Host Cell Adhesion of *Acinetobacter baumannii* DSM30011 Strain. *Frontiers in Microbiology*, **10**, 2450.

de Silva,P.M. *et al.* (2017) Effect of Incubation Temperature on Antibiotic Resistance and Virulence Factors of *Acinetobacter baumannii* ATCC 17978.

Tacconelli,E. *et al.* (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *www.thelancet.com/infection*, **18**.

Vijayakumar,S. *et al.* (2019) Accurate identification of clinically important *Acinetobacter* spp.: an update. *Future Sci. OA*, **5**, 395–2056.

Whiteway,C. *et al.* (2022) *Acinetobacter baumannii*. *Trends in Microbiology*, **30**, 199–200.

Whiteway,C. *et al.* (2021) Scarless excision of an insertion sequence restores capsule production and virulence in *Acinetobacter baumannii*. *The ISME Journal 2022*, 1–5.

Wick,R.R. *et al.* (2018) Kaptive Web: User-Friendly Capsule and Lipopolysaccharide Serotype Prediction for *Klebsiella* Genomes. *jcm.asm.org 1 Journal of Clinical Microbiology*, **56**, 197–215.

Wijers,C.D.M. *et al.* (2021) Identification of Two Variants of *Acinetobacter baumannii* Strain ATCC 17978 with Distinct Genotypes and Phenotypes. *Infection and Immunity*, **89**.

Wisplinghoff,H. *et al.* (2008) Molecular epidemiology of clinical *Acinetobacter baumannii* and *Acinetobacter* genomic species 13TU isolates using a multilocus sequencing typing scheme. *Clinical Microbiology and Infection*, **14**, 708–715.

Wyres,K.L. *et al.* (2020) Identification of *Acinetobacter baumannii* loci for capsular polysaccharide (KL) and lipooligosaccharide outer core (OCL) synthesis in genome assemblies using curated reference databases compatible with Kaptive. *Microbial Genomics*, **6**.

Zhou,Z. *et al.* (2020) The EnteroBase user's guide, with case studies on *Salmonella* transmissions, Yersinia pestis phylogeny, and *Escherichia* core genomic diversity. *Genome Research*, **30**, 138–152.