Journal of Global Antimicrobial Resistance 23 (2020) 154–161

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Genomic analysis of two *Acinetobacter baumannii* strains belonging to two different sequence types (ST172 and ST25)

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ARTICLE INFO

Article history:
Received 30 April 2020
Received in revised form 27 August 2020
Accepted 4 September 2020
Available online 20 September 2020

Keywords:
Genomic analysis
Pathogenesis
Acinetobacter baumannii
Sequence type

ABSTRACT

Objectives: Acinetobacter baumannii is an opportunistic nosocomial pathogen that is the main focus of attention in clinical settings owing to its intrinsic ability to persist in the hospital environment and its capacity to acquire determinants of resistance and virulence. Here we present the genomic sequencing, molecular characterisation and genomic comparison of two *A. baumannii* strains belonging to two different sequence types (STs), one sporadic and one widely distributed in our region.
Methods: Whole-genome sequencing (WGS) of Ab42 and Ab376 was performed using Illumina MiSeq-I and the genomes were assembled with SPAdes. ARG-ANNOT, CARD-RGI, ISfinder, PHAST, PlasmidFinder, plasmidSPAdes and IslandViewer were used to analyse both genomes.
Results: Genome analysis revealed that Ab42 belongs to ST172, an uncommon ST, whilst Ab376 belongs to ST25, a widely distributed ST. Molecular characterisation showed the presence of two antibiotic resistance genes in Ab42 and nine in Ab376. No insertion sequences were detected in Ab42, however 22 were detected in Ab376. Moreover, two prophages were found in Ab42 and three in Ab376. In addition, a CRISPR-cas type I-Fb and two plasmids, one of which harboured an AbGRI1-like island, were found in Ab376.
Conclusions: We present WGS analysis of two*A. baumannii* strains belonging to two different STs. These findings allowed us to characterise a previously undescribed ST (ST172) and provide new insights to the widely studied ST25.

1. Introduction

Acinetobacter baumannii is a significant nosocomial pathogen that is a main focus of attention in clinical settings not only due to its ability to persist in the hospital environment but also its capacity to acquire determinants of resistance and virulence, including resistance to all available families of antibiotics. In fact, *A. baumannii* is currently ranked as the first pathogen on the World Health Organization's (WHO) priority list for the development of new antibiotics [1], which shows that there is a great need for research to combat this species. In addition, according to the US Centers for Disease Control and Prevention (CDC) report '*Antibiotic resistance threats in the United States 2019*', carbapenem-resistant *Acinetobacter* was also found in the urgent threat category. Several *A. baumannii* genomes have been studied and have shown high genome plasticity driven by recombination and horizontal gene transfer events [2].

Infections caused by *A. baumannii* are increasing worldwide, possibly due to the rapid expansion of a select number of genetically distinct lineages. Whilst international clones (IC) I to III have a worldwide distribution, other successful lineages, which spread in single institutions and/or worldwide, have been

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http://dx.doi.org/10.1016/j.jgar.2020.09.006

identified [3]. For example, sequence type 10 (ST10) was recently described as the most common ST reported in Australia [4], whilst ST78 (IC-VI) and ST79 (IC-V) were highly reported in Brazil [5]. In Argentina, a recent epidemiological study demonstrated the presence of *A. baumannii* clinical isolates belonging to ST1, ST25 and ST79 [6]. Moreover, a recent surveillance of carbapenem-resistant *Acinetobacter* spp. isolates in Argentina showed the emergence of bla_{NDM-1} among *A. baumannii* isolates, all of them belonging to ST25 [7].

Here we present the genome analysis and comparison between two *A. baumannii* strains belonging to two different STs, the uncommon ST172 that has only been mentioned to date in China and Korea [8,9], and ST25 widely distributed mainly in Latin America but also in France, Italy, Libya, the United Arab Emirates and Australia [6,7,10–12]. These findings allowed us to characterise a ST not studied before and provided greater knowledge on the ST25 already widely investigated.

2. Materials and methods

2.1. Bacterial strains and antimicrobial susceptibility testing

Acinetobacter baumannii strains Ab42 and Ab376 were recovered from an endotracheal aspirate and a catheter, respectively, in 2011 at two different hospitals in Buenos Aires, Argentina. The strains were identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik). Antimicrobial susceptibility testing was performed using a VITEK®2 system (Supplementary Table S1) (bioMérieux, Marcy-l'Étoile, France). Minimum inhibitory concentrations (MICs) were interpreted using the Clinical and Laboratory Standards Institute (CLSI) categories.

2.2. Whole-genome sequencing (WGS) of Ab42 and Ab376 clinical strains and Ab42 transformant cells

Shotgun WGS was performed using an Illumina MiSeq-I system with Nextera XT libraries for sample preparation. De novo assembly was performed with SPAdes Assembler v.3.1.0 using a pre-assembly approach with Velvet [13]. The RAST (Rapid Annotation using Subsystem Technology) server was used to predict and annotate open reading frames, and BLAST v.2.0 software was utilised to validate the predictions [13]. The Whole Genome Shotgun project has been deposited in GenBank under the accession no. **JAAZUE000000000** for Ab42 and **JABACK000000000** for Ab376, respectively. The versions described in this paper are versions **JAAZUE000000000** and **JABACK000000000**.

2.3. Sequences, genome sampling, homologue identification and phylogenetic analysis

The 4881 assemblies from the *Acinetobacter* genus, including genome sequences, annotation files and protein-coding sequences, were downloaded via ftp from ftp.ncbi.nlm.nih.gov/genomes (26 October 2019) (Supplementary Table S2). Forty-seven highly conserved ribosomal protein sequences (Supplementary Table S3) were used to check the completeness of genomes (as a measure of genome quality) and to estimate evolutionary divergence among genomes. The 47 coding genes were identified in 4454 genomes using PSI-Blast with a minimum identity value of 50% and a query coverage of 75%. The 47 homologous groups of 4454 proteins were independently aligned using MUSCLE with the fastest algorithm (options: -maxiters 1 -diags1) [14]. Poorly aligned positions were deleted using Gblock software [15] with default parameters and subsequently concatenated for distance estimation using the distmat program from the EMBOSS package [16]. Genomes displaying ≥99% similarity in these conserved genes were clustered. One genome for each cluster was randomly selected for subsequent phylogenetic analysis as representative of the diversity of the genus. This resulting second data set comprises 113 sampled genomes plus the genomes of Ab42 and Ab376 reported here (sampled genomes from the genus in Supplementary Table S2). A total of 323 putative orthologous genes were found among the 115 selected genomes. The OrthoMCL method was implemented in the GET_HOMOLOGOUS software and used for homologous identification [17,18]. BLAST searches were performed with a minimal identity value of 40% and minimal query coverage of 75%. Orthologous protein sequences were aligned using Clustal Omega v.1.2.0 [19] and then concatenated for phylogenetic analysis. Evolutionary model selection and maximum-likelihood phylogenetic analysis were done using IQ-TREE 1.6.1 [20]. Ultrafast bootstrap modality was used to evaluate branch support. The phylogenetic position of Ab42 and Ab376 was established and 31 genomes from the most inclusive and robust monophyletic group were used to track back into the original first data set closely related genomes to both strains. Seventy-six closely related genomes were selected from the first data set and plus both local strains were retrieved and used for further phylogenetic analysis (closely related genomes in Supplementary Table S2). A total of 488 putative orthologous genes were identified among the 78 genomes using GET_HOMOLOGUES software as previously described. Clusters were subsequently aligned and trimmed using Clustal Omega v.1.2.0 and Gblock, respectively. Blocks were finally concatenated and a phylogenetic tree was built by means of IQ-TREE 1.6.1 as mentioned above.

2.4. Average nucleotide identity (ANI) estimation

A pairwise two-way ANI score was estimated among all 78 closely related genomes using the ani.rb script developed by Luis M. Rodriguez-R and available at enveomics.blogspot.com. The ANI index is used to delineate species from genome sequence data [13,21]. Thus, if two genomes display an ANI value of ≥95%, both strains are believed to belong to the same species. Phylogenetic analysis was used to support ANI results. Also, we confirmed the species identification using the Pasteur MLST scheme by *mlst* script (https://github.com/tseemann/mLst) [22].

2.5. Genomic comparison, gene content and sequences analysis

CARD-RGI and ARG-ANNOT software were used to identify antibiotic resistance genes [13]. ISfinder and PHAST were used to identify insertion sequence (IS) elements and phages and prophages, respectively [13]. PlasmidFinder was used to detect the presence of Enterobacteriaceae plasmids. plasmidSPAdes software, which distinguishes plasmid sequences via the read coverage of contigs, was also used [13]. The epidemiological tool MLST (Multi-Locus Sequence Typing) was used to determine the sequence type (ST) for Ab42 and Ab376 [22]. The schemes proposed by the Institut Pasteur and the University of Oxford were used. Identification of virulence genes was carried out using the Virulence Factor Database (VFDB) [23]. IslandViewer software v.4.0 was also used to identify genomic islands. The K and OC loci were analysed using Kaptive software [24].

3. Results and discussion

3.1. WGS analysis of the genomes of Ab42 and Ab376 revealed particular features in these two distinct clones

A total of 7 748 636 high-quality paired-ends reads for strain Ab42 and 2 161 512 for strain Ab376 were produced with an

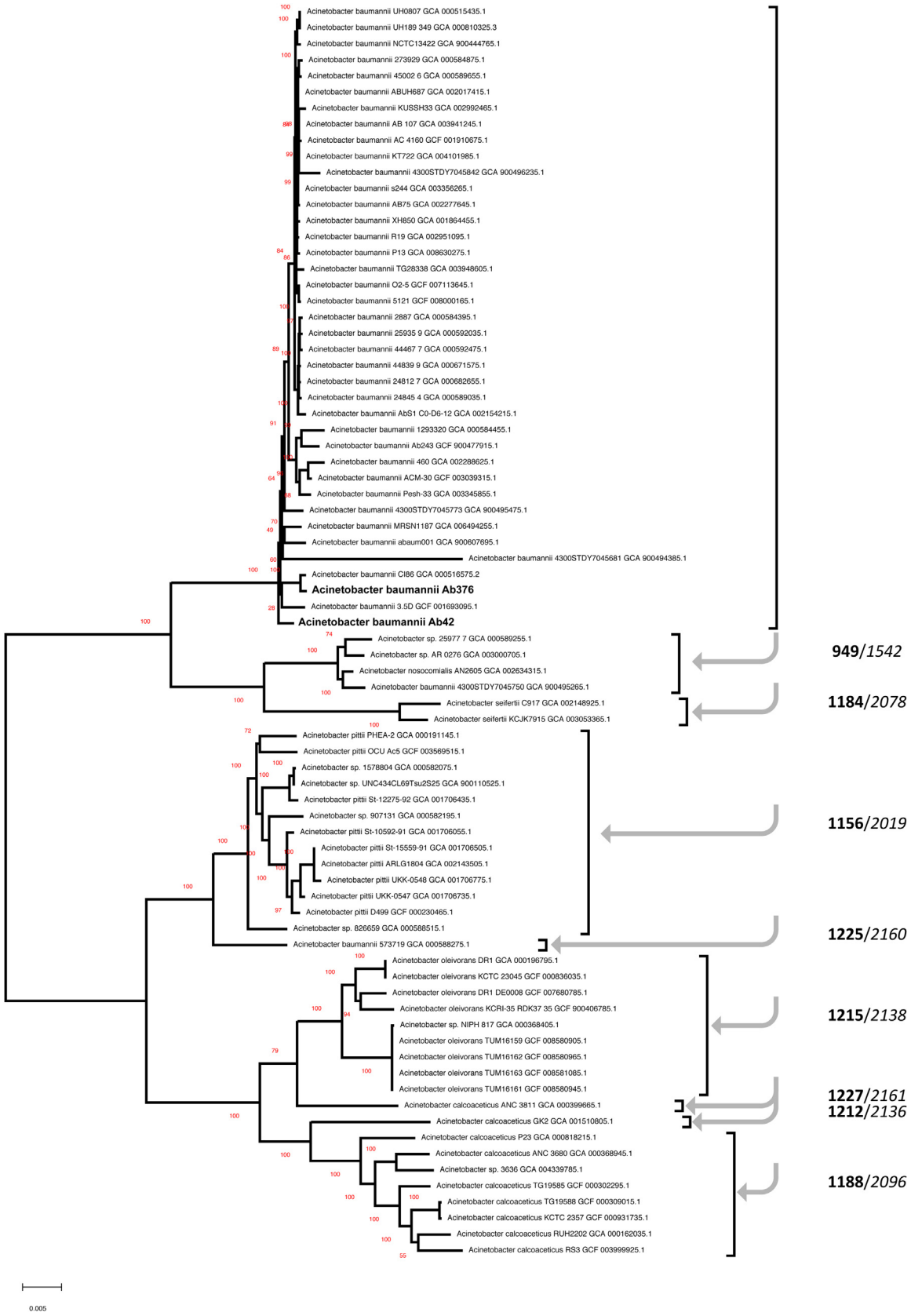

Fig. 1. Maximum-likelihood phylogenetic tree of *Acinetobacter baumannii* strains Ab42 and Ab376 and closely related *Acinetobacter* genomes. The phylogenetic tree is based on 488 concatenated orthologous proteins. The tree was inferred using IQ-TREE v.1.6.12 software. The ultrafast bootstrap approximation test was used to evaluate branch supports, which is indicated in red next to nodes. Genomes from the same species, based on two-way average nucleotide identity (ANI) score, are indicated in braces. The number of completely conserved genes (100%) and highly conserved genes (95%) between *A. baumannii* and other species is indicated in bold and italics, respectively. The assembly accession number of each genome is indicated next to the strain name (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

average size of 385 bp for Ab42 and 866 bp for Ab376. More than 98.97% of the generated reads were assembled resulting in a mean coverage of 230× for Ab42 and 119× for Ab376. Corrected reads showed an average length of 111 bp and 236 bp for Ab42 and Ab376, respectively. As expected for the genus, assemblies summed to around 3.95 Mb with an N_{50} contig size of 462 425 for Ab42 and 163 856 for Ab376 (max length 661 192) and with a G+C content of ∼39%. The Ab42 and Ab376 genomes and annotation are available at NCBI GenBank database under accession codes **JAAZUE000000000** and **JABACK000000000** for Ab2 and Ab376, respectively. A total of 3487 protein-coding genes were predicted in the genome of Ab42 and 4149 in the genome of Ab376. One complete ribosomal cluster and up to 67 tRNAs were identified in each genome.

Furthermore, the phylogenetic position of both strains among available *Acinetobacter* genomes was studied based on a previously described pipeline [25]. Phylogenetic analysis based on putative orthologous genes suggested that both described strains belong to the *A. baumannii* species (Fig. 1; Supplementary Fig. S1; Supplementary Table S4), and this result is also supported by high ANI score values (ANI score > 98% for both strains). Both strains show basal positions within the group (Fig. 1). Strain Ab42 appeared as the more basal lineage of the species, whilst Ab376 was also basal but more closely related to CI86 (BioProject PRJNA224116) and 3.5D strains (BioProject PRJNA224116); all results were very well supported by bootstrapping test. We identified 1246 highly conserved gene families among the *A. baumannii* genomes analysed, comprising the core genome. Moreover, 2094 genes were identified as the soft-core genome (n > 95% of the analysed genomes) and 27 698 as the pangenome (Fig. 1). We found that *A. baumannii* species showed a similar number of conserved genes with closely related species. Thus, the number of putative orthologous genes among closely related species was approximately 1200. Strain Ab42 had 113 unique genes and Ab376 had 367 (unique genes are those not found in any other analysed *A. baumannii* genomes) (see Supplementary Table S5 for a complete list of unique genes and sequences). Most of these unique genes were annotated as hypothetical proteins (62.8% for Ab42 and 51.5% for Ab376), phage-related proteins or mobile elements. Some gene clusters could be identified among these genes.

In addition, both isolates were analysed at an epidemiological level in order to identify the clonal lineage to which they belonged, for which purpose the MLST database for *A. baumannii* was used. The analysis revealed that Ab42 belonged to an uncommon ST (ST172) that has been described only in China and Korea [7,8]. In the PubMLST database, there is only one ST172 isolate that was recovered from Argentina [26], and only two genomes, including A42, in the GenBank database. The ST172 clustered closely to ST176, which was also described in Hong Kong [27]. Moreover, the common ancestor of both STs was ST374, previously described in Australia and Tanzania [28]. Kumburu et al. described that neither IS elements nor chromosomal resistance genes were detected in ST374 [28]. Our data would suggest an evolution of this ancestral ST to a more resistant clone. Besides, Ab376 belongs to ST25, a worldwide distributed ST and one of the most common ST that has recently emerged in Argentina. In a study performed by Rodríguez et al. in 2018, which included 100 *A. baumannii* isolates recovered from different hospitals in Argentina, ST25 was the second most prevalent ST accounting for 34% of the isolates [6]. These results were also in agreement with a previous study conducted by our group in tigecycline-resistant *A. baumannii* isolates, where the same regional epidemiological distribution was observed [29]. In addition, ST25 *A. baumannii* isolates were reported in other countries of Latin America as well as in Europe, the United Arab Emirates and Australia [6,7,10,11]. The presence of 129 ST25 genomes was found in the GenBank Database, whilst there were only 12 ST25 in PubMLST. The collected data show an increase prevalence of ST25 throughout the world, suggesting a change in the global epidemiology of *A. baumannii* major clones.

3.2. Distinct context of resistance determinants between Ab42 and Ab376

To determine the repertoire of resistance genes harboured by Ab42 and Ab376, the ARG-ANNOT and CARD-RGI databases were used. A total of two and nine resistance genes were identified in Ab42 and Ab376, respectively. The only two resistance genes found in Ab42 were the β-lactamases $bla_{\text{OXA-91}}$ ($bla_{\text{OXA-51-like}}$) and $bla_{\text{ADC-21}}$. Both genes exhibited a genetic context lacking ISs upstream or downstream of them. The $bla_{\text{OXA-91}}$ gene was found flanked upstream by a gene encoding an *N*-acetyltransferase and downstream by the *fxsA* gene, which encodes a membrane protein, as was previously reported [30,31]. The $bla_{\text{ADC-21}}$ gene was found flanked by *folE* encoding the GTP cyclohydrolase as well as a gene encoding a hypothetical protein. Ab376 harboured three β-lactamases genes ($bla_{\text{OXA-23}}$, $bla_{\text{OXA-64}}$ and $bla_{\text{ADC-25}}$), four aminoglycoside-modifying enzyme genes [*aac(3′)*-IIa, *aac(6′)*-Ian, *strA* and *strB*], a tetracycline resistance gene [*tet*(B)] and the *sul2* gene conferring sulfonamide resistance. This is consistent with what has been described in the literature about genomes belonging to ST25 carrying a large number of resistance genes [3,32,33]. Analysis of the genetic context of these genes showed an association with mobile genetic elements such as ISs (IS*Aba1*, IS*1006*, IS*1008*, IS*CR1* and IS*CR2*) and an antibiotic resistance island (AbGRI1-like). The $bla_{\text{OXA-23}}$ gene was found upstream of a gene encoding an ATPase and downstream of a truncated copy of IS*Aba1* (315 bp); this context is a truncated version of the Tn*2008* transposon. Truncated versions of Tn*2008* have also been described in the literature [34]. The $bla_{\text{ADC-25}}$ gene was found downstream of a gene encoding an outer membrane protein, and the $bla_{\text{OXA-64}}$ ($bla_{\text{OXA-51-like}}$) gene was found in the same context as described in Ab42. The tetracycline resistance gene *tet*(B) was found as part of a platform, which has been previously described by our group in minocycline-resistant isolates [35,36]. In this case, *tet*(B) was associated with IS*CR2* and the genes *strA*, *strB* and *sul2* and this platform was found as part of an antibiotic resistance island that was also present in a plasmid. This coincides with the reports of Hamidian et al. [37] and Nigro and Hall [12], where they describe the presence of these genes in plasmids, transposons and antibiotic resistance islands in *A. baumannii* ST25 isolates. Finally, the *aac(3′)*-IIa gene was flanked by the IS*1008* sequence and a transposase-encoding gene, whilst *aac(6′)*-Ian was found upstream of the IS*CR1* sequence and downstream of IS*1006* whose sequence was partial; both structures were part of the same plasmid mentioned above. In 2017, Heidary et al. described the high prevalence of aminoglycoside resistance genes in a collection of 100 clinical isolates of *A. baumannii*. Among these resistance genes, *aac(3′)*-IIa, and *aac(6′)*-Ian were found in 72% and 68% of the analysed isolates, respectively [38]. Moreover, reports in the literature describe the presence of these and other aminoglycoside resistance genes in plasmids, transposons and integrons, with the presence of aminoglycoside resistance genes being highly prevalent among *A. baumannii* resistant isolates [38,39].

The whole genome analysis exposed a difference between both genomes; whilst Ab42 harbours two resistance genes, Ab376 possesses nine on its genome. These observations are in accordance with the susceptibility profile observed among both studied strains. Ab42 was susceptible to most common antibiotics used to treat *A. baumannii* infections and also susceptible to antibiotics used in research laboratories such as kanamycin [29,35]. On the other hand, Ab376 showed an extremely drug-resistant phenotype, including resistance to ampicillin, amikacin, gentamicin, kanamycin, meropenem, imipenem, ciprofloxacin,

minocycline, fosfomycin, trimethoprim/sulfamethoxazole and ceftazidime [29].

3.3. Efflux pumps: new variants present in Ab42 and Ab376

Among the five superfamilies of efflux pumps, resistance–nodulation–division (RND) systems (AdeABC, AdeFGH and AdeIJK) are the most prevalent in *A. baumannii* [40]. The genes coding for the efflux pumps AdeABC, AdeIJK and AdeFGH, and their regulatory genes *adeSR*, *adeN* and *adeL*, respectively, were detected in Ab42 and Ab376. In both genomes, a truncated version of the AdeABC efflux pump was observed, since the *adeC* gene was not present. Marchand et al. reported that absence of the *adeC* gene in *A. baumannii* BM4454 strain did not affect resistance to various pump substrates (aminoglycosides, β-lactams, chloramphenicol, erythromycin, tetracyclines and the dye ethidium bromide), suggesting that AdeAB would be using an alternative outer membrane protein for such expulsion [41]. Sequence comparison of the AdeAB variants between Ab42 and Ab376 showed 98% and 99% amino acid identity, respectively. Also, 98% and 99% amino acid identity was observed in the comparison between AdeS and AdeR among them. Moreover, 99% amino acid identity was observed in AdeI and in the regulatory protein AdeN and 100% in AdeJK. Analysis of the efflux pump AdeFGH and the regulatory protein AdeL showed 99% amino acid identity among the genomes.

Further analysis of the sequences found in Ab42 compared with all available data showed that the AdeAB variants presented 100% amino acid identity with the sequence reported in *A. baumannii* ACICU strain (**ACC57137**) and 99% with the sequence reported in *A. baumannii* UH22908 strain (**ETQ58319**), respectively. Moreover, the AdeSR regulatory proteins had 99% amino acid identity with the sequence reported in *A. baumannii* Naval-57 strain (**ELX05382**) and 100% with that reported in *A. baumannii* WC-692 strain (**EKA67859**), respectively. Analysis of the AdeIJK efflux pump variants and the AdeN regulatory protein showed 100% amino acid identity with that reported in *A. baumannii* AB0057 strain (**CP001182**). In addition, the AdeFGH efflux pump variants showed 100% amino acid identity with the AdeF and AdeH sequences present in *A. baumannii* 99063 (**EXC52323**) and *A. baumannii* Naval-83 (AN **EKL50718**), and 99% amino acid identity with the AdeG variant present in *A. baumannii* MRSN15313 (**AYY88458**). The AdeL regulatory protein showed 100% amino acid identity with *A. baumannii* ACICU (**ACC57814**).

On the other hand, in Ab376 the AdeAB variants presented 100% amino acid identity with the sequences reported in *A. baumannii* OIFC143 strain (**EJG23435** and **EJG21428**, respectively) for both variants. The AdeSR regulatory proteins had 100% amino acid identity with the sequences reported in *A. baumannii* HEU3 strain (**PPC49758**) and *A. baumannii* OIFC143 strain (**EJG23352**) for AdeS and AdeR, respectively. Moreover, analysis of AdeIJK and AdeN showed 100% amino acid identity with the AdeI variant reported in *A. baumannii* UPAB1 (**QCR55677**) and 100% identity with the AdeJK variant reported in strain AB0057 (**ACJ41740** and **ACJ41741**). In addition, it was observed that the AdeN regulatory protein had 99% amino acid identity with that described in strain AB0057 (**ACJ42440**). Analysis of the AdeFG variants showed 100% amino acid identity with those reported in *A. baumannii* 810CP (**AXG85718** and **AXG85719**) and 99% amino acid identity for AdeH with respect to *A. baumannii* OIFC143 (**EJG18603**). The AdeL regulatory protein showed 100% amino acid identity with the sequence present in *A. baumannii* UH19608 (**ETQ47105**).

These results showed that Ab42 presented amino acid variants of AdeS, AdeB and AdeG not previously described, whilst Ab376 harboured amino acid variants of AdeN and AdeH not previously described. The relationship between mutations in AdeS and AdeN with tigecycline resistance has been mentioned in the literature. In addition, it was described that the presence of mutations in AdeS has an impact on ciprofloxacin resistance [42,43].

3.4. Virulence traits in Ab42 and Ab376

The VFDB database [23] was used to characterise the virulence traits presents in Ab42 and Ab376. A minimum amino acid identity of 30% and a coverage >75% were considered as parameters for virulence gene identification. A total of 75 and 92 virulence-related genes were determined in the genomes of Ab42 and Ab376, respectively (Supplementary Table S6). Analysis of the genes showed that for 55 (73.33%) and 71 (77.17%) of the genes found in Ab42 and Ab376, respectively, the previous association of these genes and their role in virulence were experimentally demonstrated in *A. baumannii*. Comparison between Ab42 and Ab376 genomes showed that they shared 67 genes. Among some of the virulence-associated genes identified in both genomes, we found genes encoding the type IV pili system involved in motility and biofilm formation as well as the *csu* and *pgaABCD* operons, both also involved in biofilm formation. In addition, genes encoding the PAB, BLP-1 and BLP-2 biofilm-associated proteins were also found in both genomes [44]. The type II secretion system (T2SS) and the Ata autotransporter were also found in Ab42 and Ab376. Moreover, the 13 core genes of the type VI secretion system (T6SS), considered as a virulence factor, and three of its accessory genes (*tagN*, *tagF* and *tagX*) were present in both isolates. The outer

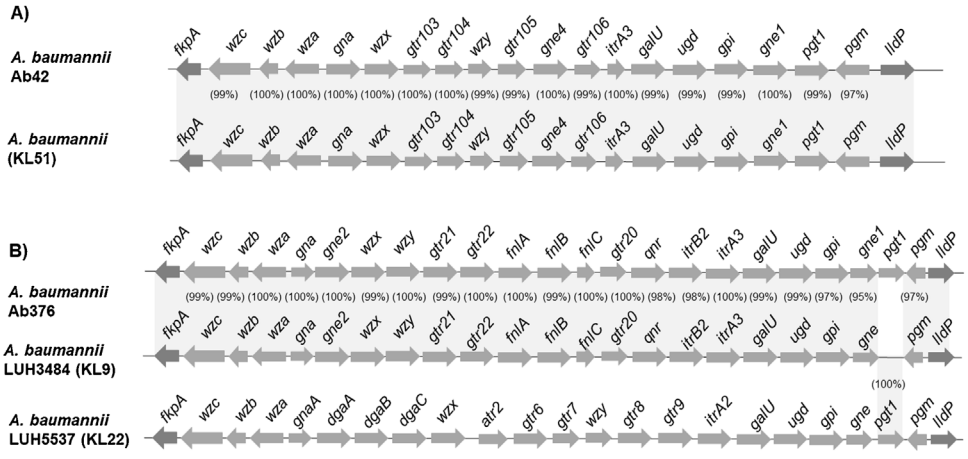

Fig. 2. Comparison of the genetic structure of the K locus responsible for the synthesis of capsular polysaccharide in (A) Ab42 and (B) Ab376, LUH3484 and LUH5537. The comparison was made using Kaptive software.

membrane protein *ompA*, which constitutes a virulence factor with multiple important effects on the pathogenesis of *A. baumannii*, was also found in Ab42 and Ab376. In addition, both genomes harboured the *bauDCEBA* locus, which is related to the transport of ferric acinetobactin within the cytoplasm, as well as the *bauF*, *barAB* and *basABCDEFGHIJ* genes.

Other important virulence factors identified in *A. baumannii* are the capsular polysaccharide biosynthesis loci [K locus (KL)] and LPS loci [OC locus (OCL)]. It has been established that these loci play a role in pathogenesis, allowing the bacteria to resist the bactericidal activity of complement and providing protection from diverse environmental conditions such as desiccation and antibiotic exposure [45–48]. As reported in the literature, the composition and structure of the K and OC loci can vary considerably between different strains of the same species; in fact, they are considered as areas of high genomic variability [47,49]. Analysis of Ab42 and Ab376 genomes allowed us to identify the presence of KL and OCL in both strains.

Analysis and comparison of the overall structure of the KL for Ab42, with a GC content percentage of 34.1%, showed 99.24% nucleotide identity and 100% coverage with KL51. This structure was also present in an *A. baumannii* strain (**MN148384**) (Fig. 2A).

In addition, the KL in Ab376, with a GC content percentage of 34.5%, showed 98.79% nucleotide identity and 99.83% coverage with KL9. This structure was also present in strain *A. baumannii* LUH3484 (**KC526895**) described by Hu et al. [46]. Moreover, we also found the *cgmA* gene as part of the same structure. Taking into account that the *pgt1* gene had not been described as part of the KL9 locus, but it had been described present in other KL loci such as in KL22, the comparative analysis also suggested that the locus present in Ab376 would be a hybrid as well (Fig. 2B).

In addition, the K locus of *A. baumannii* ST25 strains D46 and D4 has been described in the literature, whose K loci were identified as KL14 and KL16, respectively [50,51].

Furthermore, the genes that are part of the OC locus were found in both genomes. The OCL of Ab42 had a GC content of 37% and presented 97.40% nucleotide identity and 100% coverage with OCL1. This structure was also present in *A. baumannii* A91 strain (**JN968483**) (Fig. 3A). In addition, according to the classification made by Kenyon et al., this OCL would belong to group A [48]. In Ab376 the OCL had a GC content of 36.9% and showed a nucleotide identity of 99.88% and 100% coverage with OCL5. This structure was also described in strain *A. baumannii* D13 (**HM590877**). Taking into account the classification made by Kenyon et al. [48], this OCL would belong to group B (Fig. 3B).

3.5. Mobile genetic elements found in Ab42 and Ab376

Mobile genetic elements, particularly ISs, are recognised for their role in modelling the bacterial genome and concomitantly given them the capacity to adapt to multiple environments [52,53]. The role of ISs in the development of antibiotic resistance and genome plasticity in *A. baumannii* has been extensively mentioned in the literature [52,54]. Surprisingly, no ISs were detected in the Ab42 genome, however a total of 22 ISs were detected in Ab376 (11 complete and 5 in a partial version). Among the complete ISs detected, two copies of IS*17*, a copy of IS*CR1*, IS*Aha2*, IS*Vsa3*, IS*Acsp1*, IS*26*, IS*Aba14*, IS*Aba34* and IS*Aba11* were found. Two copies each of IS*CR2* and IS*Aba1* were also found (one complete and one partial each). Whilst the detected partial versions corresponded to IS*Aba12*, IS*Aba125*, IS*1006*, IS*1007* and IS*1008* (Supplementary Table S7).

Using CRISPRFinder, we found a structure compatible with a CRISPR-cas system type I-Fb in the Ab376 genome. The system had a total of 48 spacers, whose nucleotide identity was 100% with respect to the spacers described in *A. baumannii* 741,019 strain (**KM998782**) [55]. Moreover, the Cas6f, csy3, csy2, csy1, Cas3 and Cas1 genes found possessed 100% nucleotide identity with those described in strain AR_0088 (**CP027530**). According to Karah et al. [55], two types of CRISPR-cas have been described so far in *A. baumannii*, one of them being CRISPR-cas of type I-Fb. In that work, the authors analysed 76 isolates of *A. baumannii* containing CRISPR-cas type I-Fb and observed the presence of CRISPR-cas of type I-Fb mainly in isolates belonging to ST25 and ST2, which is consistent with our finding. Moreover, a CRISPR-cas of type I-Fb was also found in *A. baumannii* AYE isolate that belongs to ST1.

In addition, using PHAST tool to predict phage sequences, we identified two prophages (pf1 and pf2) in the Ab42 genome, with one of them being complete and the other incomplete, respectively (Supplementary Table S8). In pf1 and pf2, the coding genes for the head, capsid and tail were found. pf2 possessed only the specific recognition sites *attL* (TTAAAAATAAAAT) and *attR* (TTAAAAA-TAAAAT) and the prophage encoding integrase. In the Ab376 genome, three prophages (pf3–pf5), one complete and two incomplete, were found (Supplementary Table S8). pf3 harboured the genes for the head, capsid, tail and a phage integrase. In pf4 and pf5 only the specific recognition sites *attL* (TATAAAAATGAA and TTTTGCATTTTT, respectively) and *attR* (TATAAAAATGAA and TTTTGCATTTTT, respectively) and a gene encoding for a phage integrase was observed. It has been documented that the presence and/or number of prophages is not homogeneous in bacterial

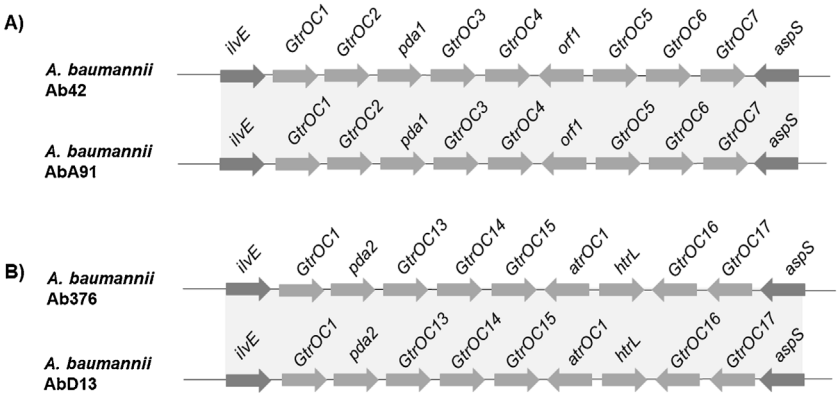

Fig. 3. Comparison of the genetic structure of the OC locus responsible for synthesis of the OC component of lipopolysaccharide in (A) Ab42 and AbA91 and (B) Ab376 and AbD13. The comparison was made using BLAST software v.2.2.31.

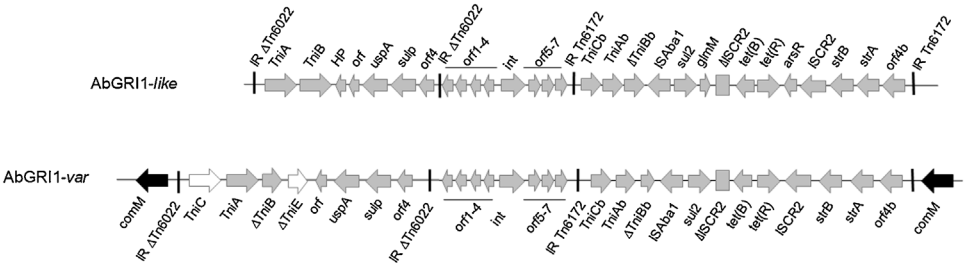

Fig. 4. Comparison of the genetic structures of the AbGRI1-like and AbGRI1-var islands in Ab376. The grey and white arrows indicate the shared and unshared genes between them.

genomes, possibly due to their intrinsic genetic variability [56,57]. This was observed in our work where we found different amounts of prophages in the genomes analysed. Moreover, it is likely that the presence of a CRISPR-cas system in Ab376 had contributed to the absence of more prophages.

We also used PlasmidFinder and plasmidSPAdes software to search for plasmids or plasmid-related sequences in both genomes. No plasmids or plasmid-related sequences were found in Ab42, whilst two plasmids were found in Ab376. Both plasmids (pAb376_1 and pAb376_2) are almost identical (99%) to previously described plasmid pAba7804b described in ST25 *A. baumannii* 7804 strain (**CP022285**) [58] and plasmid pPB364_2 described in *A. baumannii* PB364 strain (**CP040427**), respectively. pAb376_1 also harboured an AbGRI1-like island, aminoglycoside resistance genes [*aac(6′)*-Ian and *aac(3′)* II], ISs (IS*CR1*, IS*1008*, IS*1006*, IS*17*, IS*Acps1*, IS*Aba12*, IS*Aba11*, IS*Aba14*, IS*1007* and IS*Aha2*), the mercury resistance operon and the BREX system, the latter involved in phage resistance. On the other hand, pAb376_2 possessed the toxin–antitoxin system (BrnA and BrnT), the insertion sequence IS*26*, the RepAci4 replicase gene, a TonB-dependent receptor, the encoding gene for septicolicin and *mobAS* genes. Plasmid extraction using the protocol of Hansen and Olsen 1978 showed the presence of plasmids, therefore confirming the results obtained by the bioinformatics search.

Also, prediction of genomic islands (GIs) was performed using IslandViewer 4 software. Ten and six possible GIs were detected in Ab42 and Ab376, respectively. All of the GIs predicted had a deviation considered in the percentage of GC content (average of 35%) with respect to the GC content of the complete genome sequence analysed, fulfilling one of the assumptions to consider this region as a GI. Moreover, an AbGRI1-like antibiotic resistance island (Fig. 4), which has been widely reported in isolates of *A. baumannii* belonging to global clone 2 (GC2), was also identified within the Ab376 genome. This AbGRI1-like has a size of 27 442 bp and a GC content of 40.01% and particularly was not found invading the *comM* gene, which was described as a target for insertion of mobile elements, but in the pAb376_1 plasmid described above. The GI also presents a truncated version of the transposon Tn*6022* and a structure similar to Tn*6172*. Comparative analysis showed that this GI resembles the AbGRI1-var island described by Hamidian and Hall [59]. The authors suggested that the absence of genes within Tn*6022* and homologous recombination between the IS*CR2* sequence present in AbGRI1−0 and a partial copy of IS*CR2* adjacent to the *tet*(B) determinant could explain the rise of AbGRI1-Var. A similar structure to this island was found in plasmid pD4 present in an *A. baumannii* ST25 isolate [32].

4. Conclusion

The genomic analysis carried out in the present work allowed us to describe a ST not previously characterised (ST172) and provided greater characterisation of ST25 that is widely dispersed. Furthermore, we believe that the obtained results demonstrate the great ability of *A. baumannii* to constantly evolve and adapt, which allows it to be one of the opportunistic human pathogens with the greatest clinical success worldwide.

Funding

The authors' work was supported by NIHSC3GM125556 to MSR. JSF was supported by grant MHIRT2T37MD001368 from the National Institute on Minority Health and Health Disparities, National Institutes of Health.

Ethical approval

Not required.

Conflict of interests

None declared.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2020.09.006.

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