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Genomic insights of *Acinetobacter baumannii* ST374 reveal wide and increasing resistome and virulome

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ABSTRACT

WGS-based surveillance has significantly improved the ability to track global spread and emergence of multidrug-resistant clones of clinically relevant pathogens. In this study, we performed the genomic characterization and comparative analysis of an *Acinetobacter baumannii* (strain Ac56) belonging to the sequence type ST374, which was isolated for the first time in Brazil, in 1996. Genomic analysis of Ac56 predicted a total of 5373 genes, with 3012 being identical across nine genomes of *A. baumannii* isolates of ST374 from European, Asian, North and South American countries. GoeBURST analysis grouped ST374 lineages into clonal complex CC3 (international clone IC-III). Resistome analysis of ST374 clone predicted genes associated with resistance to heavy metals and clinically relevant beta-lactams and aminoglycosides antibiotics. In this regard, in two closely related *A. baumannii* strains, the intrinsic bla_{ADC} gene was linked to the insertion sequence IS*Aba1*; including the Ac56 strain, where it has been possibly associated with intermediate susceptibility to meropenem. Other four carbapenem-resistant *A. baumannii* strains carried the IS*Aba1*/bla_{OXA-23} gene array, which was associated with the transposon Tn*2008* or with Tn*2006* in an AbaR4-type resistance island. While most virulence genes were shared for *A. baumannii* strains of ST374, three isolates from Thailand harbored KL49 capsular loci, previously identified in the hypervirulent *A. baumannii* LAC-4 strain. Analysis of thirty-four predicted plasmids showed eight major groups, of which GR-6 (LN−1) and GR-2 (LN−2) were prevalent. All strains, including the earliest isolate Ac56 harbored at least one complete prophage, whereas none CRISPR-associated (*cas*) gene was detected. In summary, genomic data of *A. baumannii* ST374 reveal a potential of this lineage to become a successful clone.

1. Introduction

A. baumannii has emerged as an important opportunistic pathogen responsible for serious infections, mainly in critical ill patients, linked with high mortality rates (Sharifipour et al., 2020; Son et al., 2020). Currently, *A. baumannii* was the most common pathogen associated with viral-bacterial co-infections in patients infected with SARS-CoV-2 submitted to invasive mechanical ventilation in intensive care units (ICUs) (Lai et al., 2021; Sharifipour et al., 2020; Shinohara et al., 2021).

The emergence and establishment of some *A. baumannii* clones in hospital settings have been supported by their remarkable ability to develop multidrug resistance and escape from the hosts' defenses, with long-term persistence on abiotic surfaces (Antunes et al., 2014; Leal et al., 2020; Lodi et al., 2019); whereas their notable genomic plasticity favors a promptly adaptation to hostile environment conditions by advantageous mutations, genomic rearrangement by recombination, and

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exchanging of mobile genetic elements (MGEs) associated with resistance genes and virulence factors (Diancourt et al., 2010; Leal et al., 2020).

The molecular epidemiology of *A. baumannii* has showed the prevalence of three clonal lineages among clinical isolates of multidrug-resistant (MDR) *A. baumannii* collected worldwide, corresponding to international clones (IC) I to III (Karah et al., 2012). In Europe and Asia, most of MDR *A. baumannii* clinical isolates belong to IC-I and IC-II, corresponding to clonal complexes CC1 and CC2, respectively (Holt et al., 2016; Karah et al., 2012). In Brazil, *A. baumannii* isolates have been clustered within the clonal complexes CC1/IC-I, CC15/IC-IV, CC25/IC-VII and mainly from the South American endemic CC79/IC-V clonal complex (Camargo et al., 2020; Medeiros and Lincopan, 2013), which have been associated with the production of acquired OXA-carbapenemases. However, the *A. baumannii* CC3/IC-III had not been documented so far, in this country.

Based on a comparative analysis with publicly available ST374 genomes, the present study aimed to report the complete genome of the earliest clinical isolate of *A. baumannii* ST374 described worldwide, being the first in Brazil, and to describe the genetic features of resistance and virulence present in this lineage.

2. Materials and methods

2.1. Microbiological and genomic analysis of A. baumannii

During a retrospective surveillance and monitoring antimicrobial resistance program conducted by the Medical Microbiology Laboratory (MML) of the State University of Maringá in South Brazil, a MDR *A. baumannii* strain (Ac56), which was recovered from tracheal secretion in 1996, represented the earliest *A. baumannii* isolate belonging to ST374. From records, the Ac56 isolate initially exhibited an intermediate susceptibility to meropenem (MIC 4 mg/L) identified by automated system (MicroScan, Dade Behring, West Sacramento, CA, USA) at the hospital of origin. Minimum inhibitory concentrations (MIC) of meropenem and polymyxin B were confirmed in the MML by broth microdilution method (CLSI, 2018) and results were interpreted using both CLSI and EUCAST breakpoints (CLSI, 2019; EUCAST, 2021). Molecular typing methods performed in the MML (PFGE, ERIC-PCR) have been used for the investigation and control of local outbreaks of nosocomial infection (van Belkum et al., 2007). Based on this methods, Ac56 isolate showed high similarity (>0.93 using Bionumerics® v. 6.5 software) to representatives of an endemic cluster of other MDR *A. baumannii* clinical isolates (Brondani Moreira et al., 2018; dos Santos Saalfeld et al., 2009; Viana et al., 2011), which were recently associated with an outbreak in an COVID-19-specific ICU (Shinohara et al., 2021). Sequence type ST374 was determined by multilocus sequence typing (MLST) analysis according to the Institute Pasteur scheme (Jolley et al., 2018). In this regard, nucleotide sequences were obtained using an ABI 3730 DNA Analyzer (Life Technologies, Carlsbad, CA). The WGS of Ac56 was performed using the Nextera DNA Flex library preparation and NextSeq 500 platform (Illumina, Inc., San Diego, CA). Sequences were assembled by Unicycler, annotated by PROKKA and deposited into NCBI GenBank under the accession number WPIQ01000000.

2.2. Comparative genomic analyses

For comparative analysis, eight publicly available genomes of *A. baumannii* of ST374 were retrieved from NCBI GenBank database (https://www.ncbi.nlm.nih.gov) (Table S1). The genome of the strain NCTC13301 ST374 was used as reference. The Roary software was used to estimate the pan-genome of the ST374 clade, their core and accessory genomes of each strain applying a blastp identity threefold of 95% (Page et al., 2015). The selected genome sequences had the assembled contigs reordered and aligned by Mauve tool (Rissman et al., 2009) to visual analyses of the scaffold genomes using as reference the NCTC13301 strain, which had the genome sequenced by a long-read sequencing technology and assembled into three contigs presumed to correspond to the complete chromosome (UIFZ01000003) and two plasmids (UIFZ01000001 and UIFZ01000002). Phylogenetic relationships between the ST374 and representative *A. baumannii* strains were estimated by CodonTree method based on single-copy core genes using RAxML tool (Randomized Axelerated Maximum Likelihood) and the phylogenetic tree was built by Maximum likelihood tree estimation at web-based PATRIC tool (Wattam et al., 2017). The population structure based on the *A. baumannii* MLST database of Pasteur scheme was determined by goeBURST v1.2.1 (http://www.phyloviz.net/).

Plasmids were *in silico* identified by BLAST searching for known plasmid replication proteins (Rep proteins) groups (GRs) (Bertini et al., 2010) and the contigs manually analyzed to identification of plasmid maintenance, mobilization and conjugation modules (Salgado-Camargo et al., 2020).

Analyses of acquired resistance genes and virulence determinants were performed by the ResFinder v4.1 (Zankari et al., 2012) and VFanalyzer searching (http://www.mgc.ac.cn/VFs/), respectively. The outer core *locus* (OCL) and capsule *locus* (KL) were defined using the Kaptive tool (https://kaptive-web.erc.monash.edu/) and Bautype tool (http://bautype.net/Acinetobacter_baumannii/home/). Insertion sequences (IS) elements were manually annotated according to the ISfinder database (https://www-is.biotoul.fr/). The PHASTER (PHAge Search Tool - Enhanced Release; https://phaster.ca/) was used to identify putative phages within the bacterial genomes and CRISPRCas-Finder for putative clustered regularly interspaced short palindromic repeats (CRISPR) identification (https://crisprcas.i2bc.paris-saclay.fr/) (Arndt et al., 2016; Couvin et al., 2018).

3. Results and discussion

The Ac56 strain exhibited a MDR phenotype to piperacillin-tazobactam, ceftazidime, cefepime, ciprofloxacin, levofloxacin, amikacin and gentamicin (Magiorakos et al., 2012). The isolate was classified as intermediate to meropenem (MIC = 4 mg/L) and susceptible to polymyxin B (MIC = 2 mg/L) according to the CLSI and EUCAST breakpoints. MLST analysis confirmed that Ac56 belonged to ST374. Based on goeBURST analysis, the ST374 was found as a locus variant of clonal complex CC3 (international clone IC-III) (Fig. S1), which was grouped *A. baumannii* lineages from European, North American, South American, and Asian countries (Huang et al., 2012; Levy-Blitchtein et al., 2018; Opazo-Capurro et al., 2019). In addition, this CC3 included MDR isolates of ST374 carrying the carbapenemase gene $bla_{\text{OXA-23}}$ from hospital of Thailand (Loraine et al., 2020) and United Kingdom (NCTC 3000 project; https://www.sanger.ac.uk/resources/downloads/bacteria/nctc/).

The ST374 isolates showed an expanding pan-genome, whereas the number of core genes remained relatively stable (Fig. S2 A). The size of the pan-genome was 5373 genes, with 3012 (56%) being part of the core-genome. From the accessory genes, a number of 1135 genes was found in at least a pair of strains (shell genes) and 1226 genes were only found in a single strain (cloud genes) (Fig. S2 B,C). Overall, the Ac56 and NCTC13301 strains shared the highest phylogenetic relationship also regarding the structure and number of harbored plasmids. The MRSN11816 strain was the most distant one compared to all ST374 *A. baumannii* strains (Fig. 1).

Virulome prediction identified genes involved in biofilm (*algC*, *bap* and *pgaABCD* locus) and *pili* formation (*csu*), adherence (*carO* variant IV and *ompA* porins), lipid A synthesis (*lpxABCDLM* locus), biosynthesis of the LPS core (*lpsB*), serum resistance (*pbpG*), phospholipase C and D (*plc* and *plcD*), LysR-type transcriptional regulator as well as acinetobactin genes cluster (*bau, bas*, *bar*, *ent*) in most *A. baumannii* ST374 genomes (Fig. 1). Efflux pump associated genes of the resistance-nodulation-cell division (RND: *adeAB*, *adeFGH* and *adeIJK*) and multidrug and toxic compound extrusion (MATE: *abeM* and *abeS*) types, including the

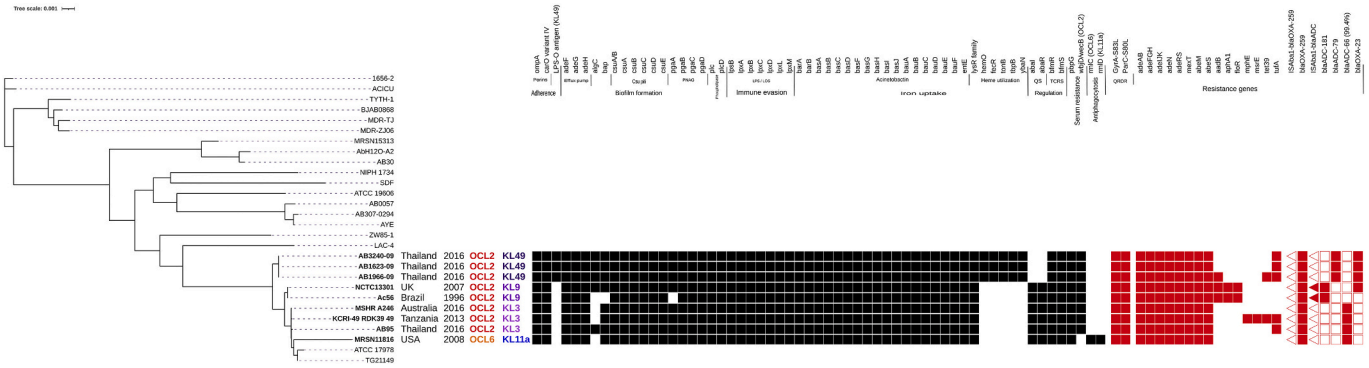

Fig. 1. Overview of the resistance and virulence genes identified as well as definition of the OC/KL loci for the *Acinetobacter baumannii* ST374 strains. The strains are sequentially ordered according to their phylogenetic relationship (left panel). The identification of the resistance genes was carried out with the sequences from the ResFinder database and the virulence genes were identified in the VFDB database. Additionally, *carO* and *algC* genes were detected using BLAST. A positive match in the figure is indicated by a colored shape. Negative matches are shown as blank spaces. In the figure, the genes are sorted by antibiotic classes to which they confer resistance and mechanistic features that they likely confer to *A. baumannii* strains.

regulators (*adeN*, *adeSR* and *mexT*), were found in the totality of included ST374 genomes. The *hemO* gene cluster encompassing an extra cytoplasmic function (ECF) sigma factor (*fecI*); its cognate anti-sigma factor (*fecR*); TonB-dependent receptor (*tbpB*) and membrane protein (*tonB*); tonB-related protein; an inner membrane protein (*ybaN*); hypothetical proteins; and a heme oxygenase (*hemO*) for iron acquisition was identified among the AB3240–09, AB1623–09 and AB1966–09 strains from Thailand and has been identified in most of hypervirulent *A. baumannii* clinical strains, including the LAC-4 (Giardina et al., 2019; Ou et al., 2015). Furthermore, OCL and KL locus of these strains were highly similar to OCL2 and KL49, respectively. The KL49 was recently showed being associated with increased host mortality in a mouse pneumonia model. Finally, with the exception of the quickly distant MRSN11816 strain, which presented an OCL6 and a KL11a locus, the remaining studied ST374-strains have an OCL2 loci and KL-type varying among KL9 or KL3 following the phylogenetic relationship of these strains (Fig. 1).

Genes for resistance to aminoglycosides [(*aphA1* (*aph(3′)-Via*) and *aadB* (*ant(2″)-Ia*)], phenicol (*floR*), macrolide (*mph*E, *msr*E), tetracycline (*tet39*), mocimycin (*tufA*) and beta-lactams (bla_{ADC}, bla_{OXA}) were identified among the ST374 *A. baumannii* genomes (Fig. 1). QRDR mutations producing the S83L substitution in GyrA and the S80L in ParC were found in all included strains. The class C beta-lactamase $bla_{ADC\text{-}181}$ gene was found associated with upstream IS*Aba1* element in Ac56 and NCTC13301 strains, which has been implicated in the overexpression of $bla_{ADC\text{-}like}$ genes and decreased susceptibility to beta-lactams, including cephalosporins and carbapenems (Héritier et al., 2006; Jeon et al., 2014).

Carbapenem resistance in *Acinetobacter* is mostly due to the carbapenem-hydrolyzing class D β-lactamases (CHDLs), especially by acquired OXA-carbapenemases, such OXA-23, −143, −24/40, −58 families, and/or overexpression of OXA-51-like CHDLs, when its encoding gene is associated with upstream IS*Aba1*-like elements (Karah et al., 2012). All ST374 strains analyzed harbored the $bla_{OXA\text{-}259}$ allelic variant of the $bla_{OXA\text{-}51}$–family-gene, but none associated with IS*Aba*-like elements.

Global epidemiology studies have identified OXA-23-producing *A. baumannii* belonging to CC2 (IC-II) and CC1 (IC-I), and sporadically belonging to CC3 (IC-III) (Holt et al., 2016; Levy-Blitchtein et al., 2018). Among ST374 clones analyzed in this study, the $bla_{OXA\text{-}23}$ gene was identified in the NCTC13301 genome onto Tn*2008* element inserted in a hypothetical coding region between the *hemH* and *murI* genes in the chromosome of the NCTC13301 strain (Fig. 2B); whereas in AB3240–09, AB1623–09 and AB1966–09 strains the $bla_{OXA\text{-}23}$ gene was carried by a Tn*2006* element, bearing by a TnAbaR4-type genetic island integrated into disrupted chromosomal *comM* gene (Fig. 2B). The spread of $bla_{OXA\text{-}23}$ has been driven by horizontal gene transfer through MGEs, including the most prevalent composite transposons Tn*2006* and Tn*2008* as also found in these three *A. baumannii* ST374 OXA-23-producers (Chen et al., 2017; Nigro and Hall, 2015).

None acquired OXA-carbapenemase gene was identified in the AB95 strain. However, its *comM* gene was interrupted by an TnAbaR genomic island, partially assembled into two contigs (Fig. 2C). One of them containing the Tn*6664* element, probably as the AbaR-backbone and the other contig with the sequence compatible to an Integrative and Conjugative Element (ICE) and/or a conjugative transposon. The *comM*-AbaR-like genomic island in AB95 seems to have been built by independent and successive DNA recombination events, despite the fragmented assembling of that locus. The Ac56, NCTC13301 and MRSN11816 strains had an intact *comM* gene locus.

Another variable genomic region of the ST374 *A. baumannii* strains was found as an ICE inserted in a tRNA-Ser gene of the NCTC13301, Ac56 and KCRI-49 RDK39_49 strains, but absent in the other genomes. These ICE is flanking by an integrase (*int*) gene and corresponds to a defense (genomic) island (DI) including genes involved in different defense processes, such as putative Abortive Infection (AbI), Restriction-modification (R-M), BacteRiophage EXclusion (BREX) and Toxin/Antitoxin (T/A) DarTG systems, anti-phage mechanisms of the bacterial defense arsenal (Fig. 2D). The NCTC13301 and Ac56 strains present an additional cluster of genes to putative proteins containing HTH (helix-turn-helix), WYL-domain and NYN (Nedd4-BP1, YacP-like Nuclease) domains, whose are predicted to be ligand-sensing that could bind to nucleotides or nucleic acid fragments to regulate defense systems such as the abortive infection AbiG system (Lopatina et al., 2020). Lastly, the KCRI-49 RDK39_49 strain has a second ICE (42.5 kb in length) in a vicinity region containing genes to hypothetic and putative phage-relative proteins (Fig. 2D). The DIs seems to accumulate diverse genes to mechanism of bacterial defense against bacteriophages and certainly play an important role on *A. baumannii* adaptation and survival (Bernheim and Sorek, 2020).

About the *A. baumannii* plasmids, they have been classified into a small number of plasmid lineages (LN), whose members of a LN share a common or a very closely related DNA replication initiator protein (Rep), classified into Rep groups (GR); and frequently the replication and maintenance modules and other genes demonstrating a strong ancestral relationship between the plasmids of a LN (Salgado-Camargo et al., 2020). According to the phylogenetic analysis of the Rep-proteins plasmids (Fig. S3 and Table S2), we could identify 16 known and six probable novel Rep-proteins among the ST374 genomes. The ST374 presented at least two to up to seven plasmid replicons *per* strain. Plasmid-replicons of LN-1 (GR6) and LN-2 (GR2) from these ST374 strains were most common.

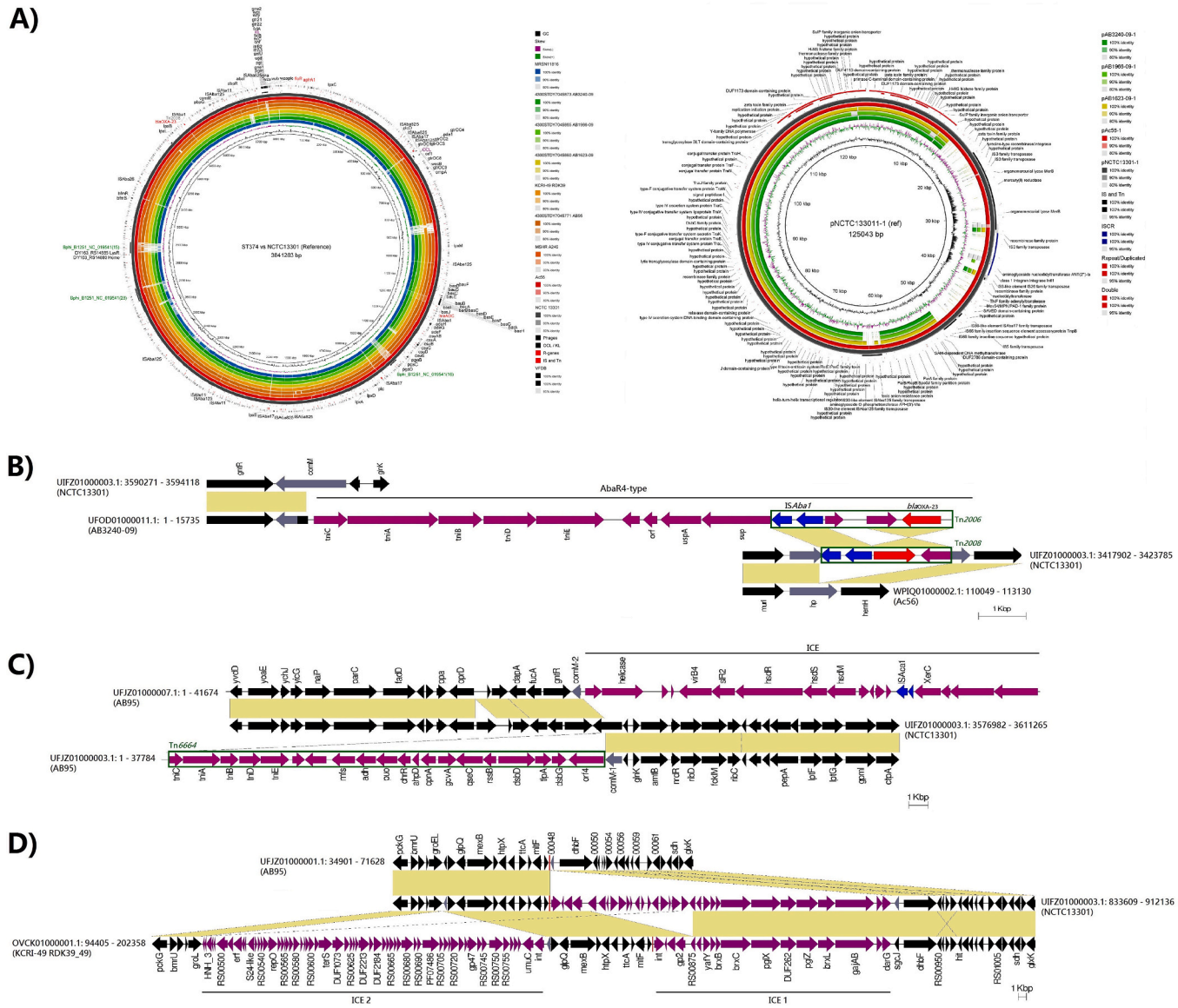

Fig. 2. Genomic characteristics of *Acinetobacter baumannii* ST374 strains. (A) Genome comparison of nine ST374 *A. baumannii* genomes using NCTC13301 chromosome as the reference genome (left panel) and five plasmids aligned with closely related pAbaNCTC13301–1 (right panel). Rings are color coded for different strains and their respective plasmids. The innermost two rings represent GC content and GC skew. The position of locus OC and KL (purple), resistance related genes (red), virulence genes, phage region (green) and mobile elements are indicated on the outermost ring representing the NCTC 13301 chromosome. The outer circle contains mobile elements (black), repeated region (red), and IS*CR* element (blue) of pAbaNCTC13301–1 as well as the results of the genome annotation process. (B) Comparison and genetic organization of the transposons: Tn*2006* in AB3240–09 (UFOD01000011.1) strain and Tn*2008* in the chromosome of NCTC 13301 (UIFZ0100003.1) strain. The $bla_{\text{OXA-23}}$ gene (red) flanked by an insertion sequence IS*Aba1* (blue) is located within both transposons. The green boxes represented the coverage of the transposon region. (C) Schematic representation of Tn*6664* genetic structure in AB95 (UFJZ01000003.1) strain *comM* gene fragments are indicated in gray arrows and green boxes also representing the coverage of the transposon region. In AB95 contig UFJZ01000007.1 was identified putative genes for DNA metabolism and recombination by DNA double-strand break and repair, such as genes for type I restriction-modification system polypeptides; a putative DNA repair protein RecN; bipolar DNA helicase HerA; ATPase-dependent endonuclease and other putative DNA-binding proteins, flanked by a site-specific tyrosine recombinase XerC encoding gene and an IS*Aca1* element (blue). (D) Comparison and genetic organization of the ICE inserted in a tRNA-Ser gene (red bar) of the NCTC13301 (UIFZ01000003.1) and KCRI-49 RDK39_49 (OVCK01000001.1) strains. Gray arrows indicated the disrupted regions in the genomic region. The ICE 1 into these three strains contain an *intA* (integrase), *alpA* (AlpA family phage regulatory protein), *Gp2* (PF11726, inovirus-type Gp2 protein), DY163_RS05340 (helix-turn-helix transcriptional regulator) genes followed by the BREX system, which includes *pglZ* (phosphatase), *pglX* (methyltransferase) and *brxABCL* genes that encodes an RNA-binding anti-termination protein (BrxA), an unknown protein (BrxB), an ATP-binding protein (BrxC) and a protease (BrxL), as well as the toxin-antitoxin system DarTG (*darTG*). Most of the genes related to the ICE 2 in the KCRI-49 RDK39_49 strain are coding hypothetic and putative phage-relative proteins. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The sequences of two plasmids were *in silico* identified in the reference strain NCTC13301 (~125 kb and 55 kb in length) and in the Ac56 strain (>68 kb and 55 kb), belonging to LN-1 (repAci6; GR6) and to an orphan lineage of *A. baumannii* plasmids (Table S2). The 125 kb-plasmid of NCTC13301 presented a duplicated region, both containing genes predicted as replicase encoding genes belonging to the same GR6 group. It might be associated with sequencing artifacts since plasmids with duplicate replication machinery are highly unstable and rapidly eliminated from a population. Nonetheless, the *in silico* translation into deduced amino acid sequences revealed a frame-shifted Rep-protein

gene, similar to GR6-P group found in the pACICU2 plasmid, probably, due to the co-integration of two plasmids resulting in a dysfunctional replication module. Plasmids belonging to LN-1 were also identified in the Ac56 (68.7 kb), AB1623–09 (72 kb), AB1966–09 (72 kb) and AB3240–09 (72 kb) draft genomes, with 99% of identity to the LN-1-plasmids pAbaNCTC13301–1 (125 kb) and pA85–3 (CP021787.1; 86.3 kb), quickly varying on the general plasmid structure and their predicted proteome among these plasmids (Fig. S3).

These LN-1 plasmids presented the Zeta-toxin/anti-toxin (TA) system and the putative *traGHF*, *trbC*, *traWVBKELCUN* genes clusters involved in plasmid maintenance and conjugation (Table S2 and Fig. S3). LN-1 is one of the few *A. baumannii* plasmid lineages that has large sets of conjugation genes and have been shown to be able to easily transfer by conjugation. It might facilitate the wider distribution of the LN-1 among the ST374 and other *A. baumannii* lineages. In general, the structure of LN-1 plasmids containing the replication, partition and maintenance mechanisms has been very stable over time, considering the geographical and temporal distance of them. Differences on LN-1 (GR6) plasmid identified among these ST374-strains were found in both pNCTC13301–1 and pAbaAc56–1 plasmids (Fig. 2A; right panel). The presence a Tn*21*-like structure comprising the mercuric resistance (*mer*) operon; an IS*CR* element flanked by an IS*26* elements, containing a class 1 integron with the aminoglycoside-2″-adenyltransferase (*aadB*) gene in the cassette region flanked by an IS*1006* element. The *floR* gene was associated to an IS*3* family element and the aminoglycoside 3′-phosphotransferase gene (*aphA1*) was found surround by IS*Aba125* elements in these plasmids of the Ac56 and NCTC13301 strains. While IS*Aba12* (IS*5*-family) and IS*Aba17* (IS*66*-family) were nearby a cluster of genes to a thiamine biosynthesis enzyme, adenosine deaminase and uncharacterized proteins.

The plasmids of an orphan lineage that was identified from the strains NCTC13301 (pAbaNCTC13301–2; 55.3 kb) and Ac56 (pAbaAc56–2; 55 kb) presented most of the predictable proteins as hypothetical or functionally uncharacterized; but the replication, maintenance and stability genes were present, such as an ungrouped RepB-family plasmid replication initiator protein (Pfam01051; Rep_3); Phd/YefM and YoeB TA-modules and a putative ParA partition system, but none predictable IS or resistance genes were found in these plasmids (Table S2).

The Ac56 (13.7 kb) and the AB1623–09 (8.8 kb), AB3240–09 (8.8 kb), AB1966–09 (8.8 kb) and AB95 (1 kb; *rep* gene fragmented) strains had contigs comprising genes for a GR2 RepE replication protein; the SplTA-module and a putative gene for septicolysin, highly comparable to the pORAB01–3 plasmid (15.1 kb) that fall into LN-2 lineage of *A. baumannii* plasmids and carries the $bla_{\text{OXA-23}}$ gene flanked by IS*Aba1* elements (Fig. S3). Additionally, AB1966–09 strain carried a LN-4 plasmid of about 5 kb (pAbaAB1966-09-3, UFOB01000015.1) bearing the aminoglycoside-resistance *aadB* gene in this strain, similar to LAC-4 plasmid pABLAC2 (CP007714.1); a predicted RepA2 protein encoding gene, likely belonging to a family of proteins for rolling-circle replication (RCR) associated to a putative mobilization protein (MobA/MobL) and the TA-module genes of RelE/ParE family was found in a 4.5 kb assembled contig (pAbaAB1966-09-4, UFOB01000016.1) and supposed to be of a small RCR-plasmid. A Rep protein from GR16 group was identified in the AB3240–09, AB1623–09 and AB1966–09 strains, while the remaining identified plasmid were harbored uniquely by a single strain (Fig. S3).

The presence of prophages was investigated among the ST374 *A. baumannii* strains. Prophages are often blamed for the insertion/acquisition of new genes by bacteria, however this invasion can be prevented with the presence of CRISPRs that was also investigated among the studied strains (Westra et al., 2012). Using the reordered scaffold of the ST374 draft genomes based on the complete genome of NCTC13301 strain as reference, the PHASTER tool predicted thirty prophage sequences from these genomes of which thirteen regions were classified as "intact"; nine regions as "incomplete" and eight regions as "questionable" (Table S3). Our analysis revealed the presence of different variants/lineage of prophages among the genomes analyzed, which is possibly due to their intrinsic genetic variability (Bobay et al., 2013). Among the structures predicted to be "intact", the presence of the integrase as well as its specific recombination sites *att* were found, except the region 1 in AB95 strain that did not present integrase. We observed a linkage of ten such prophages to the genus *Acinetobacter*, while the other three were linked to the genera *Salmonella*. Seventeen defective prophages categorized as "questionable" and "incomplete" were described in the genus *Acinetobacter* (14 regions) and from Enterobacteriacea (3 regions). Finally, none CRISPR-associated (*cas*) gene was detected in the ST374 genomes by CRISPRCasFinder. This observation indicated the high susceptibility of ST374 strains towards phage infection. Visual analysis and localization of the prophage sequences using the Mauve tool is shown in the Fig. S4.

When this manuscript was being written the complete genome of a ST374 *A. baumannii* strain DT0544C (SAMN14833494) was published in the NCBI, from a New-Delhi metallo-beta-lactamase (NDM)-producing clinical isolate, collected in 2017 from Tanzania and close-related to the KCRI-49 RDK39_49 strain on resistome and other genes repertory (data not shown). The DT0544C strain have plasmid with the new proposed replication group GR34 (55.3 kb in length), as same as Ac56 and NCTC13301 strains; and a 3.9 kb-plasmid containing genes for conjugation and mobilization modules (TraD and MobA/MobL-family proteins, respectively), but non-predicted replication-protein.

4. Conclusions

Genomic comparative analysis of the earliest *A. baumannii* ST374 described worldwide and reported in this study (Ac56 isolate in 1996 from Brazil) with other eight publicly available genomes belonging to the ST374 from four different continents reveal us worrisome data. Recent isolates, such those three collected in 2016 from Thailand, carrying an even broader arsenal of virulence and resistance determinants, may demonstrate the potential capacity of this lineage for genetic recombination to become prospectively a successful clone.

Data availability

This Whole Genome Shotgun project of Ac56 isolate has been deposited at DDBJ/ENA/GenBank under the accession WPIQ00000000 (BioProject: PRJNA592456; BioSample: SAMN13427462; SRA number: SRR11859021). The version described here is the first version, WPIQ01000000.

Founding sources

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Author contributions

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Declaration of Competing Interest

All authors report no conflicts of interest relevant to this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2021.105148.

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