The Complete Genome and Phenome of a Community-Acquired Acinetobacter baumannii

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Abstract

Many sequenced strains of Acinetobacter baumannii are established nosocomial pathogens capable of resistance to multiple antimicrobials. Community-acquired A. baumannii in contrast, comprise a minor proportion of all A. baumannii infections and are highly susceptible to antimicrobial treatment. However, these infections also present acute clinical manifestations associated with high reported rates of mortality. We report the complete 3.70 Mbp genome of A. baumannii D1279779, previously isolated from the bacteremia infection of an Indigenous Australian; this strain represents the first community-acquired A. baumannii to be sequenced. Comparative analysis of currently published A. baumannii genomes identified twenty-four accessory gene clusters present in D1279779. These accessory elements were predicted to encode a range of functions including polysaccharide biosynthesis, type I DNA restriction-modification, and the metabolism of novel carbonaceous and nitrogenous compounds. Conversely, twenty genomic regions present in previously sequenced A. baumannii strains were absent in D1279779, including gene clusters involved in the catabolism of 4-hydroxybenzoate and glucarate, and the A. baumannii antibiotic resistance island, known to bestow resistance to multiple antimicrobials in nosocomial strains. Phenomic analysis utilising the Biolog Phenotype Microarray system indicated that A. baumannii D1279779 can utilise a broader range of carbon and nitrogen sources than international clone I and clone II nosocomial isolates. However, D1279779 was more sensitive to antimicrobial compounds, particularly beta-lactams, tetracyclines and sulphonamides. The combined genomic and phenomic analyses have provided insight into the features distinguishing A. baumannii isolated from community-acquired and nosocomial infections.

Introduction

Acinetobacter baumannii is a significant nosocomial pathogen [1], known for its high intrinsic and laterally acquired resistance to antimicrobials [2,3] as well as its persistence on various abiotic surfaces [4-6]. The complete genome sequences of ten A. baumannii strains have been determined to date: 1656-2 [7], AB0057 [8], AB307-0294 [8], ACICU [9], ATCC 17978 [10], AYE [11], MDR-TJ [12], MDR-ZJ06 [13], SDF [11] and TCDC-AB0715 [14]. Nine of these are nosocomial isolates, whereas A. baumannii SDF was isolated from a human body louse [11]. These genome sequences have demonstrated extensive divergence due to the acquisition and accretion of various mobile genetic elements, particularly those contributing to antimicrobial resistance [15]. One mobile element of clinical import is the A. baumannii antibiotic resistance island (AbaR), that encodes resistance to a multitude of antibiotics and heavy metals [15].

Research regarding A. baumannii has occurred primarily within the context of the clinical milieu, with little known about potential environmental reservoirs of this organism. Several non-nosocomial niches of A. baumannii have been identified, including human lice [16,17], hydrocarbon contaminated soils [18,19], the plant rhizosphere [20,21] and estuaries [22,23]. A. baumannii is also known to exist outside the hospital environment as a commensal of the skin [24] and nasopharynx [25] of humans. This organism is also a public health issue outside of the hospital setting, in the form of A. baumannii infections [26,27]. A. baumannii infections [27,28] but resulting in mortalities ranging from 30–62% [27–30]. These infections are also antimicrobial susceptible [26,30] and present a more acute clinical manifestation [30], but are thought not to be reservoirs of nosocomial outbreaks [26].

The majority of CA-AB infections occur in individuals with underlying comorbidities, who reside in tropical and subtropical climates [28]. Incidences of CA-AB infection have been reported within various regions of the Asia Pacific such as Taiwan [31], Hong-Kong [30], Singapore [32], Korea [33] and Australia [34]. To a lesser extent, CA-AB infections have also been observed in non-tropical regions [27] and in otherwise healthy children and adults [35–39]. Indigenous Australians in the Northern Territory...
are overrepresented relative to the general population in rates of community-acquired bacteraemic pneumonia caused by *A. baumannii* and other pathogens [29,34]. This disparity has been attributed to the interaction of both monsoonal climate and a high prevalence of comorbidities in the indigenous Australian population including alcoholism, diabetes mellitus, chronic obstructive pulmonary disease and cigarette smoking [25,34,40].

To explore the underlying basis of epidemiological and phenotypic differences between nosocomial and community-acquired strains of *A. baumannii*, we determined the complete genome sequence of the CA-AB isolate D1279779 and phenotypically profiled this strain using phenotype microarrays. The genome and phenotype of D1279779 was subsequently compared to completely sequenced nosocomial *A. baumannii* strains.

**Materials and Methods**

**Bacterial strains, culture conditions and genomic DNA extraction**

The *A. baumannii* strain D1279779 was kindly provided by the Menzies School of Health Research (Darwin, Australia). This strain was isolated in 2009 from a bacteraemic infection of an indigenous Australian male at the Royal Darwin Hospital, where the identity and antimicrobial susceptibilities of this isolate were previously determined with a ViTIVE 2 System (bioMérieux). *A. baumannii* D1279779 was cultured in lysogeny broth (LB) and lysogeny broth agar (LBA) (both without glucose) at 37 °C. Genomic DNA was extracted with the Wizard Genomic DNA Purification Kit (Promega) from 1 mL of overnight culture as per the manufacturer’s protocol.

**DNA sequencing, genome assembly and annotation**

*A. baumannii* D1279779 genomic DNA was prepared and sequenced by 454 FLX pyrosequencing (Roche Diagnostics) at the Ramaciotti Centre for Gene Function Analysis (University of New South Wales, Sydney). The sequences reads were assembled de novo with MIRA [41] using the default parameters. The ninety-four contiguous sequences of D1279779 were reordered relative to the ten currently complete genomes of *A. baumannii* using MAUVE [42]. The highest level of syntenic was observed with the *A. baumannii* ACICU genome [9], which was subsequently utilised as a reference in Projector2 [43] to design oligonucleotides for gap closure by PCR and sequencing. Amplicons for gap closure were generated using AccuPrime™ Fx Mastermix (Invitrogen) or GoTaq DNA Polymerase (Promega) as per the manufacturer’s protocol, with variations in the annealing temperature (45°C to 65°C) and extension time (2 to 12 min) according to estimated gap sizes. The resultant amplicons were purified using the QIAquick PCR Purification Kit (Qiagen) and cloned into pGEM-T Easy (Promega), or directly sequenced using bidirectional dideoxysequencing performed by the Macquarie University DNA Analysis Facility (Sydney, Australia). The resultant chromatograms were edited and assembled in ChromasPro (Technelyum Pty. Ltd.) and crosschecked against the D1279779 genome by use of the BLASTN [44] application integrated into BioEdit [45]. The presence and directionality of the six rRNA operons in the genome was confirmed by amplification of the gap and sequencing of small junctions flanking this region. The genome assembly was finalised with the aid of CLC Sequence Viewer 6 (CLCbio). Genome annotation was conducted with the RAST automated annotation engine [46] and manual curation was performed with the aid of Artemis [47]. UGENE was routinely utilised for genome browsing and analysis [48]. The nucleotide sequences of the D1279779 chromosome and the plasmid pD1279779 have been deposited into GenBank with the accession numbers CP003967 and CP003968 respectively.

**Comparative genomics and accessory element identification**

*A. baumannii* D1279779 was compared to the ten published *A. baumannii* genomes by a reciprocal BLASTP [49] search to identify putative orthologs at an e-value cutoff of 10^-5. Trinucleotide composition of the DNA sequence was computed by the χ² analysis using a 2000 bp sliding window with a 1000 bp overlap [50]; regions containing χ² values  ≥ 500 were suggestive of atypical trinucleotide composition. Evidence derived from BLASTP and χ² analysis was used to identify regions of genomic plasticity (RGPs) [51,52], defined as either any putative mobile genetic element or contiguous cluster of genes present in D1279779 and three or less strains. Genes clusters absent in D1279779 but present in more than three other strains were also considered RGPs. The identity of insertion sequences present in the D1279779 genome were elucidated with BLASTP searches conducted in ISFinder [53].

The phylogenetic relationship of D1279779 to sequenced *A. baumannii* strains was inferred with a bootstrapped neighbour-joining analysis in MEGA5 [54] based on the concatenated nucleotide sequences of six of the seven reference genes utilised in the multilocus sequence typing (MLST) of *A. baumannii* [55]. The *fisC* gene was excluded from this analysis as it displayed atypical trinucleotide composition in D1279779, suggesting potential lateral acquisition of this gene.

**Phenotype microarray testing**

The phenomes of *A. baumannii* D1279779 and three nosocomial strains, ACICU, ATCC 17978 and AYE, were assayed with the Biolophenotype MicroArray™ (PM) system [56] to identify compounds that could serve as sole carbon (PM1-2; 190 compounds) or nitrogen sources (PM3; 95 compounds). Additionally, sensitivities to stress conditions (PM9-10; 192 conditions) and various antimicrobials compounds (PM11-20; 240 antimicrobials) were also investigated. All phenotypic tests were performed as per the manufacturer’s protocol, except cryogenic stocks of *A. baumannii* were streaked onto either LBA medium (PM1-2 and PM9-20) or Reasoner’s 2A agar (Diço) (PM3). The bacterial suspension for nitrogen source testing was supplemented with D-xylene as the sole carbon source at a concentration of 20 mM. Following inoculation, all PM plates were incubated in an OmniLog reader (Bioloph) aerobically at 37 °C for 48 h. Reduction of the tetrazolium-based dye (colourless) to formazan (violet) was monitored and recorded at 15 min intervals by an integrated charge-coupled device camera. The resultant data were analysed with the supplied manufacturer’s software, resulting in a time-course curve for colorimetric change equating to respiration rate. The phenotypes were classified on the basis of the maximal curve height; a phenotype was considered positive if the height was greater than 115 and 101 OmniLog units for nitrogen sources and all other phenotypes, respectively. Data that exceeded these cutoff values as the result of contamination from certain compounds was excluded from analysis. Observed phenotypic differences between the strains were linked to differences in genotype through a combined analysis of the EcoCyc [57], MetaCyc [58], and KEGG [59] metabolic databases and additional literature searching.

**Independent confirmatory testing of phenotype microarray data**

Five millilitres of M9 minimal media (Sigma-Aldrich), supplemented with varied carbon source compounds (20 mM), was generated using AccuPrime™ Pfx Mastermix (Invitrogen) or GoTaq DNA Polymerase (Promega) and monitored and recorded at 15 min intervals by an integrated charge-coupled device camera. The resultant data were analysed with the supplied manufacturer’s software, resulting in a time-course curve for colorimetric change equating to respiration rate. The phenotypes were classified on the basis of the maximal curve height; a phenotype was considered positive if the height was greater than 115 and 101 OmniLog units for nitrogen sources and all other phenotypes, respectively. Data that exceeded these cutoff values as the result of contamination from certain compounds was excluded from analysis. Observed phenotypic differences between the strains were linked to differences in genotype through a combined analysis of the EcoCyc [57], MetaCyc [58], and KEGG [59] metabolic databases and additional literature searching.
inoculated with a single colony of *A. baumannii* D1279779 or ACICU previously streaked on LBA medium. Cultures were incubated with shaking at 37 °C for 24 h, with the observation of turbidity from cellular replication deemed to signify a positive phenotype. A minimum of three temporally distinct replicates were performed for each tested compound, in addition to substrate and inoculation negative controls. The carbon sources tested were: L-arabinose, bromosuccinic acid, (±)-carnitine hydrochloride, L-
carnitine hydrochloride, disodium fumarate, α-D-glucose, L-histidine monohydrochloride monohydrate, polysorbate 80, putrescine dihydrochloride, L-pyroglutamic acid, quinic acid, sodium 4-hydroxybenzoate, sodium acetate trihydrate, trisodium citrate dihydrate, sodium D-gluconate, and D-xylose. All chemicals were sourced from Sigma-Aldrich (purity ≥96%), dissolved in sterile distilled water and filter-sterilised. Phenotypic testing of α-D-glucose and D-gluconic acid utilisation was further conducted in inoculation fluid zero (IF-0) [56] (without sodium pyruvate and tetrazolium dye). Further experiments with α-D-glucose were supplemented with 10 μM methoxatin disodium salt (pyrroloquinoline quinone) with the corresponding negative control.

Table 1. Comparative genome features of Acinetobacter baumannii D1279779.

| Strain     | D1279779 | ATCC 17978 | ACICU | AYE |
|------------|----------|------------|-------|-----|
| Size (base pairs) | 3704285 | 397647 | 3904116 | 3936291 |
| Plasmids   | 1        | 2          | 2     | 4   |
| G+C content (%) | 39.00   | 38.94 | 39.03 | 39.40 |
| Protein-coding sequences (CDS) | 3388 | 3787 | 3670 | 3607 |
| Insertion sequences | 18 | 14 | 14 | 33 |
| Average gene length | 935 | 888 | 929 | 951 |
| Coding regions (%) | 85.60 | 84.50 | 84.87 | 86.13 |
| tRNAs | 65 | 69 | 64 | 72 |

doi:10.1371/journal.pone.0058628.t001

Results and Discussion

Genomic features

The complete genome of A. baumannii D1279779 was determined and found to consist of a 3704285 bp circular chromosome and a plasmid of 7416 bp, dubbed pD1279779. A total of 3479 genes were annotated on the chromosome including 65 tRNAs, 6 rRNA operons and 3388 predicted protein coding sequences (CDS) (Table 1, Figure 1), which included 1019 annotated CDS (30%) predicted to encode hypothetical proteins. The plasmid pD1279779, unlike many previously sequenced A. baumannii plasmids, does not encode any insertion sequences or genes involved in antimicrobial resistance [60]. Plasmid pD1279779 appears to be of mosaic origin, with a replication repA gene sharing 100% nucleotide sequence identity with the A. baumannii plasmid p203 [60] and a 2609 bp segment sharing 99% nucleotide identity with a region from the otherwise unrelated organic peroxide resistance plasmid pMAC of A. baumannii ATCC 19606 [61].

The phylogeny and synteny of A. baumannii

The phylogeny of A. baumannii D1279779, with respect to other sequenced A. baumannii isolates, was inferred using a MLST approach [55]. The allelic profile of this strain is 12-37-2-3-2-14, but it does not match any previously assigned STs; the closest allelic profile in the MLST database belonged to an ST117 isolate (12-37-2-2-9-2-14), differing only in the recA allele. This MLST-based analysis suggested that the nearest phylogenetic relatives of D1279779 were A. baumannii strains of the international clonal (IC) lineage II, though this strain did not fall within either the A. baumannii ICI or ICII lineages (Figure 2). This observation was congruent with our own previous PCR typing, which indicated this strain did not belong to any of the three major A. baumannii lineages [62]. The phylogenetic relationship of D1279779 to other A. baumannii strains was also consistent with the notion that

![Figure 2. Phylogenetic lineage of Acinetobacter baumannii.](image-url)
community-acquired isolates are epidemiologically distinct from nosocomial isolates [26].

Genomic alignments using MAUVE indicated that *A. baumannii* D1279779 shares a high degree of genome synteny with other completely sequenced strains of *A. baumannii*, with the exception of *A. baumannii* SDF (Figure S1), which is known to have undergone both extensive genome reduction and rearrangement [11]. In *A. baumannii* D1279779, a 50.8 kb region of sequence situated between the first and sixth rRNA operons was inverted relative to other *A. baumannii* genomes (Figure 3). We confirmed this rearrangement in D1279779 by PCR analysis conducted on the original cryogenic stock. The inversion of this region resulted in reversing the orientation of a number of critical housekeeping genes as well as the origin of chromosomal replication (oriC) (Figure 3). It is of note that the opposing directionality of both rRNA operons flanking the inversion was maintained, which would otherwise potentially result in replicative blockage [63]. Presumably this genomic rearrangement was mediated by homologous recombination between the two oppositely oriented rRNA operons [64]. Such rRNA operon-mediated rearrangements in genome architecture have been known to occur in other organisms and between more distal rRNA operons [64,65], resulting in even larger inversions than observed here.

**The core and accessory genome of *A. baumannii***

The predicted proteome for all currently complete genomes of *A. baumannii* was compared by means of a reciprocal BLASTP search, which enabled estimation of the *A. baumannii* core genome size. The numbers overlaid on the phylogenetic tree in Figure 2 (adjacent to the brackets) indicate the various sizes of the core genome. A total of 1944 predicted CDS are shared between all *A. baumannii* genomes (Figure 3), which is known to have undergone both extensive genome reduction and rearrangement [11]. In *A. baumannii* D1279779, a 50.8 kb region of sequence situated between the first and sixth rRNA operons was inverted relative to other *A. baumannii* genomes (Figure 3). We confirmed this rearrangement in D1279779 by PCR analysis conducted on the original cryogenic stock. The inversion of this region resulted in reversing the orientation of a number of critical housekeeping genes as well as the origin of chromosomal replication (oriC) (Figure 3). It is of note that the opposing directionality of both rRNA operons flanking the inversion was maintained, which would otherwise potentially result in replicative blockage [63]. Presumably this genomic rearrangement was mediated by homologous recombination between the two oppositely oriented rRNA operons [64]. Such rRNA operon-mediated rearrangements in genome architecture have been known to occur in other organisms and between more distal rRNA operons [64,65], resulting in even larger inversions than observed here.

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### Table 2. Accessory elements present in the *Acinetobacter baumannii* D1279779 genome.

| (D1279779) | CDS (ABD1) | Size (kb) | G+C (%) | Putative function/features of interest | Integrate (target) | ATC* | A. baumannii orthologues |
|------------|------------|-----------|---------|---------------------------------------|-------------------|------|--------------------------|
| RGP01      | 00530–00630 | 12.1      | 31.3    | polysaccharide biosynthesis            | –                  | Y    | ABTJ_03749-03760         |
| RGP02      | 00790–00890 | 12.5      | 39.0    | unknown                               | –                  | N    | variable region           |
| RGP03      | 01090–01140 | 5.9       | 36.8    | metabolism                            | –                  | N    | ABTJ_03697-03701         |
| RGP04      | 04780–04960 | 16.6      | 41.2    | fatty acid biosynthesis                | –                  | N    | ACICU_00517–00535        |
| RGP05      | 05270–05690 | 47.3      | 37.3    | genomic island, type I restriction-modification, metabolic augmentation | Y (duxA)          | Y    | unique                   |
| RGP06      | 06860–07920 | 5.7       | 30.4    | unknown                               | –                  | Y    | unique                   |
| RGP07      | 08570–08650 | 10.0      | 33.8    | cryptic genomic island                 | Y (ND³)           | N    | unique                   |
| RGP08      | 09940–10530 | 50.6      | 38.4    | prophage, phosphoethanolamine transferase, ISAba13_2 | Y (ND³)           | Y    | pAba10,6 all ex. AB0057, ATCC 17978, SDF |
| RGP09      | 10860–10930 | 1.7       | 37.5    | lipoproteins                          | –                  | N    | unique                   |
| RGP10      | 11180–11850 | 48.0      | 38.9    | prophage, DNA polymerase V            | Y (lysC)          | N    | A1S_1142-75 AB57_1310-1224 |
| RGP11      | 12360–12570 | 19.3      | 32.9    | temperature shock, metabolism         | N                  | Y    | All ex. SDF              |
| RGP12      | 13160–13230 | 9.4       | 37.0    | unknown, ISAba13_4                    | –                  | N    | unique                   |
| RGP13      | 13580–13650 | 6.2       | 32.9    | unknown, ISAba13_5                    | –                  | N    | unique                   |
| RGP14      | 15000–15150 | 16.0      | 33.7    | genomic island, fimbriae biogenesis    | Y* (ND³)          | Y    | pAba10,6 all ex. A. baumannii |
| RGP15      | 17780–17800 | 5.0       | 25.9    | unknown                               | –                  | Y    | unique                   |
| RGP16      | 17790–17970 | 3.0       | 32.1    | unknown                               | –                  | N    | unique                   |
| RGP17      | 18030–18070 | 3.6       | 40.5    | degradative enzymes                   | –                  | N    | unique                   |
| RGP18      | 18340–18480 | 14.9      | 37.1    | fatty acid metabolism                 | –                  | Y    | A1S_1813-22              |
| RGP19      | 22050–22070 | 2.1       | 33.9    | unknown                               | –                  | N    | A1S_2209-11              |
| RGP20      | 22680–22710 | 3.4       | 38.6    | RNA modification                      | –                  | N    | A1S_2271-73              |
| RGP21      | 23900–24060 | 20.7      | 38.7    | degradative enzymes                   | –                  | N    | A1S_2397-2414            |
| RGP22      | 28390–28410 | 2.9       | 30.5    | polysaccharide biosynthesis           | –                  | N    | A1S_2896-3841            |
| RGP23      | 31330–31680 | 35.0      | 35.5    | fosfomycin resistance, ISAba13_17     | –                  | Y    | variable region          |
| RGP24      | 32600–32660 | 7.0       | 26.3    | unknown                               | –                  | Y    | A1S_3899-3901            |

*ATC - atypical trinucleotide composition; RGPs with x² values greater than 500.
*ND - Undetermined integrate target site.
*Accessory element designation in A. baumannii ACICU [9].
*Premature stop in integrate protein

doi:10.1371/journal.pone.0058628.t002
also encodes an allantoicase (Alc) which functions in a similar manner to AllC, except degradation of allantoate is performed by a single-step catalytic mechanism, rather than a dual-step one.

Two other RGPs, D1279779_RGP01 and D1279779_RGP22, encode enzymes involved in capsule polysaccharide biosynthesis. The RGP D1279779_RGP03 encodes additional copies of three enzymes (diaminopimelate decarboxylase, a ribulose-phosphate 3-epimerase, and an uroporphyrinogen decarboxylase) encoded elsewhere in the core genome.

The D1279779 genome encodes two putative prophages, one of which encodes paralogues of the error-prone DNA polymerase V subunits UmuC and UmuD; these genes are frequently associated with prophages and other mobile genetic elements such as genomic islands [74]. The second prophage encodes epimerase, and a uroporphyrinogen decarboxylase) encoded (arsenic and mercury) [2].

beta-lactams, sulphonamides, tetracyclines) and heavy metals (arsenic and mercury) [2].

A. baumannii has previously been associated with the accretion of multiple insertion sequences and genomic islands [77,78]. When present, AbaRs are capable of encoding resistance to a multitude of antibiotics (aminoglycosides, beta-lactams, sulphonamides, tetracyclines) and heavy metals (arsenic and mercury) [2]. A. baumannii D1279779 has an intact comM gene and lacks an AbaR island, which may partially explain the antimicrobial susceptibility phenotype observed for this CA-AB isolate.

Nineteen other gene clusters, present in the genomes of other A. baumannii isolates, are also absent from D1279779. Ten of the missing gene clusters are unique to A. baumannii strains of the ICI and ICII lineages. Six other gene clusters absent in D1279779 were replaced with RGPs unique to this strain or additionally present only in ATCC 17978 (Table 2, Table 3). Some of the absent genes included those involved in the β-ketoacidipate pathway [79], the D-glucarate degradation pathway [80] and potentially, the catabolism of carnitine and vanillate (Table 3). The loss of these metabolic genes very likely correlates with the observed inability of this strain to utilise glutarate, glucarate and 4-hydroxybenzoate as carbon sources but does not prevent the utilisation of carnitine (see below).

Table 3. Accessory elements absent in the Acinetobacter baumannii D1279779 genome.

| RGP_   | D1279779 boundaries | ACICU CDs    | Putative function/features of interest                  | No. of strains with RGP present |
|--------|---------------------|--------------|--------------------------------------------------------|---------------------------------|
| D01    | 02020 (comM)        | AB57_0243-0306 | A. baumannii antibiotic resistance island             | 9/10 strains AbaG1 in SDF       |
| D02    | 05890–06790         | 00685–00701   | glucose dehydrogenase 2, various insertion sequences   | 7/10 strains exclusive to ICI    |
| D03    | 09790–09710         | 00873–00880   | haem degradation, iron acquisition                       | 5/10 strains                    |
| D04    | 11106–11110         | AB57_1176–1215 | glucarate degradation pathway, vanillate degradation    | 3/10 strains mediated by ISAba13_3 |
| D05    | 12530–12580         | AB57_1379–1406 | degraded prophage                                       | 3/10 strains exclusive to ICI   |
| D06    | 13150–13240         | 01295–01313   | type VI secretion system                                | 9/10 strains replaced with D1279779_RGP12 |
| D07    | 13750–13660         | 01334–01403   | [β-ketoacidipate pathway, 2-aminoethylphosphonate transport/ metabolism, carnitine degradation] | 9/10 strains replaced with D1279779_RGP13 |
| D08    | 17690–17960         | 01810–01823   | pilin biogenesis, saccharopine dehydrogenase, monoamine oxidase | 8/10 strains exclusive to ICI and ICII |
| D09    | 17770–17810         | 01833–01838   | unknown                                                | 5/10 strains exclusive to ICI replaced with D1279779_RGP15 |
| D10    | 17920–17980         | 01852–01863   | unknown                                                | 5/10 strains Exclusive to ICI replaced with D1279779_RGP16 |
| D11    | 18190–18270         | 01892–01897   | demethylmenaquinone methyltransferase, phosphoglycerate dehydrogenase | 5/10 strains exclusive to ICI mediated by ISAba13_3 |
| D12    | 18390–18490         | 01914–01920   | metabolism                                             | 7/10 strains replaced with D1279779_RGP18 |
| D13    | 18590–18610         | 01937–01949   | various dehydrogenases                                 | 5/10 strains exclusive to ICII   |
| D14    | 20390–20400         | 02140–02235   | prophase                                               | 4/10 strains                    |
| D15    | 21340–21350         | ABK1_1354–1403 | prophase                                               | 3/10 strains                    |
| D16    | 21980–21990         | AB57_2539–2553 | two component regulation, fatty acid modification      | 3/10 strains exclusive to ICI   |
| D17    | 22190–22200         | AB57_2575–2579 | unknown                                                | 3/10 strains exclusive to ICII   |
| D18    | 23890–24070         | 02595–02623   | transcriptional regulation, transporters/permeases     | 7/10 strains replaced with D1279779_RGP21 |
| D19    | 24530–24540         | AB57_2901–2906 | crispr-associated proteins                             | 3/10 strains exclusive to ICII   |
| D20    | 28500–28510         | 03158–03160   | hypothetical proteins                                   | 4/10 strains                    |

*3' comM fragment not present in A. baumannii ACICU.
1Gene cluster not present in A. baumannii ACICU.
2Number of currently completely sequenced A. baumannii strains other than D1279779.
3DOI:10.1371/journal.pone.0058628.t003

doi:10.1371/journal.pone.0058628.t003

Also noteworthy is the presence of a genomic island (Table 3), a drug resistance island present in the majority of published A. baumannii genomes. AbaRs are characteristically found inserted within the comM gene [76], and associated with the accretion of multiple insertion sequences and genomic islands [77,78].

As with other species, D1279779 encodes an allantoicase (Alc) which functions in a similar manner to AllC, except degradation of allantoate is performed by a single-step catalytic mechanism, rather than a dual-step one [79].

Two other RGPs, D1279779_RGP01 and D1279779_RGP22, encode enzymes involved in capsule polysaccharide biosynthesis. The RGP D1279779_RGP03 encodes additional copies of three enzymes (diaminopimelate decarboxylase, a ribulose-phosphate 3-epimerase, and an uroporphyrinogen decarboxylase) encoded elsewhere in the core genome.

The D1279779 genome encodes two putative prophages, one of which encodes paralogues of the error-prone DNA polymerase V subunits UmuC and UmuD; these genes are frequently associated with prophages and other mobile genetic elements such as genomic islands and plasmids [74]. The second prophage encodes a parologue of lipid A phosphoethanolamine transferase (EptA) which facilitates the covalent modification of lipid A. Overexpression of eptA in A. baumannii has previously been associated with increased colistin resistance [25]. However, strain D1279779 appears to exhibit greater colistin sensitivity than nosocomial isolates (discussed below).

Notably, A. baumannii D1279779 does not encode the AbaR genomic island (Table 3), a drug resistance island present in the majority of published A. baumannii genomes. AbaRs are characteristically found inserted within the comM gene [76], and associated with the accretion of multiple insertion sequences and genomic islands [77,78]. When present, AbaRs are capable of encoding resistance to a multitude of antibiotics (aminoglycosides, beta-lactams, sulphonamides, tetracyclines) and heavy metals (arsenic and mercury) [2]. A. baumannii D1279779 has an intact comM gene and lacks an AbaR island, which may partially explain the antimicrobial susceptibility phenotype observed for this CA-AB isolate.

Nineteen other gene clusters, present in the genomes of other A. baumannii isolates, are also absent from D1279779. Ten of the missing gene clusters are unique to A. baumannii strains of the ICI and ICII lineages. Six other gene clusters absent in D1279779 were replaced with RGPs unique to this strain or additionally present only in ATCC 17978 (Table 2, Table 3). Some of the absent genes included those involved in the β-ketoacidipate pathway [79], the D-glucarate degradation pathway [80] and potentially, the catabolism of carnitine and vanillate (Table 3). The loss of these metabolic genes very likely correlates with the observed inability of this strain to utilise glutarate, glucarate and 4-hydroxybenzoate as carbon sources but does not prevent the utilisation of carnitine (see below).
Comparison of known or predicted virulence genes amongst A. baumannii sequences indicated strain D1279779 lacks several genes conserved amongst previously sequenced A. baumannii strains. These include a type VI secretion system gene cluster [81] (Table 3), a gene encoding the Acinetobacter trimeric autotransporter protein (Ata) [82] (which is truncated in D1279779), haem acquisition (Table 3) and a gene encoding for the biofilm-associated protein (Bap) [83,84]. The loss of these genes may correlate with the observation that D1279779 has only a modest capacity for biofilm formation and adherence to nasopharyngeal cells [62]. Nevertheless, this strain still carries other potential virulence-associated genes, including those coding for acinetobactin biosynthesis [85], capsular polysaccharide polymerisation/export [86], type I and type IV pili biogeneses [87] and phospholipases C and D [88,89].

Multiple copies of ISaba13 are present in the genome

Eighteen copies of the transposon ISaba13, previously identified in A. baumannii AB0057 [8], are present throughout the D1279779 chromosome (Figure 1). All insertion sequences, with the exception of one, are of an identical isoform (Table 4). The two insertion sequence isoforms have nucleotide identities of 99% and 96% to the ISaba13 in AB0057. Thirteen copies of ISaba13 are inserted within annotated genes, including some potentially encoding virulence and competence functions such as a fimbrial adhesin, a type I secretion domain protein, bacterial capsule synthesis protein and competence-damaged induced protein CinA (Table 4). One of the copies of ISaba13 replaced an approximately 44 kb region, present in some other A. baumannii strains, that carries genes for the catabolism of glucarate, galactarate and vanillate (Table 3, Table 4).

The catabolic phenome of A. baumannii D1279779

Biolog Phenotype MicroArrays are a respiration-based assay system that can test up to 2000 phenotypic traits simultaneously [56,90]. This system uses 96 well plates with each well testing a separate phenotype using a tetrazolium dye that produces a colour change in response to cellular respiration. The phenome of A. baumannii D1279779 was investigated with the Biolog Phenotype MicroArray System and compared with an ICI strain (AYE), an ICI strain (ACICU) and ATCC 17978, a nosocomial isolate from 1951, predating the emergence of the major global clonal lineages as the dominant nosocomial strains.

The four A. baumannii strains tested were similar in their utilization of sole carbon (Figure 4) and nitrogen sources (Figure S2). They utilized a combined total of 80 carbon sources out of the 190 tested, encompassing a range of amino acids, carboxylic acids, saccharides and miscellaneous compounds (Figure 4). Strains D1279779 and ATCC 17978 were able to utilise a greater breadth of sole carbon and nitrogen sources compared to ICI and IICII strains, particularly in relation to amino acids including alanine, asparagine, isoleucine, glutamate and homoserine (Figure 4, Figure S2). The observed phenotypic profiles suggest the emergence of ICI and IICII lineages in nosocomial settings has coincided with a narrowing of their substrate utilisation capabilities. Furthermore, A. baumannii ACICU displayed a higher respiration rate on substrates arginine, ornithine, phenylalanine, pyroglutamic acid, quinic acid and ribonolactone (Figure 4), suggesting a possible specialization in terms of carbon utilization preferences.

In order to independently confirm the Biolog respiration data, the ability of A. baumannii D1279779 and ACICU to grow on minimal media in the presence of fifteen sole carbon compounds

Table 4. Genomic coordinates of ISaba13 copies and orthologous genes disrupted.

| ISaba13_ | Coordinates | Gene(s) disrupted | Annotation/putative function | A. baumannii orthologue(s) |
|-----------|-------------|-------------------|------------------------------|---------------------------|
| 1         | 583114–584152 | ABD1_05170 | TetR family transcriptional regulator | ACICU_00556 |
| 2         | 1142807–1143845 | ABD1_10420 | prophage-associated hypothetical | ACICU_01061 |
| 3         | 1214764–1213726 | Loss of ~44 kb | glucarate/galactarate/vanillate catabolism | AB57_1176-1212b |
| 4         | 1421673–1422711 | ABD1_13230; 13240 | hypothetical protein, ankyrin repeat-containing protein | no orthologue; ACICU_01314 |
| 5         | 1463547–1464585 | ABD1_13650 | hypothetical protein | no orthologue |
| 6         | 1487979–149017 | ABD1_13850 | competence-damage inducible protein CinA | ACICU_01424 |
| 7a        | 1544569–1545607 | ABD1_14440 | MFS transporter | ACICU_01479 |
| 8         | 1787573–1788611 | – | – | – |
| 9         | 1892223–1891185 | ABD1_17670 | fimbrial family protein | ACICU_01810 |
| 10        | 1963590–1964628 | ABD1_18260 | type 1 secretion C-terminal target domain | ACICU_01891 |
| 11        | 2095736–2094698 | – | – | – |
| 12        | 2195861–2194823 | – | – | – |
| 13        | 2233207–2234245 | – | – | – |
| 14        | 2256425–2257463 | – | – | – |
| 15        | 2640973–2642011 | ABD1_24300 | hypothetical protein | ACICU_02649 |
| 16        | 2879635–2878597 | ABD1_26440 | bacterial capsule synthesis protein | ACICU_02936 |
| 17        | 3422518–3423556 | ABD1_31460 | hypothetical protein | ACICU_03597 |
| 18        | 3599602–3600640 | ABD1_33030 | hypothetical protein | ACICU_03597 |

*ISaba13 sequence isoform 2.
1gene(s) not present in A. baumannii ACICU.
1Not present in any currently complete A. baumannii genome.
doi:10.1371/journal.pone.0058628.t004
was tested. Turbidity consistent with cellular replication was observed in all tested carbon sources except for D-gluconic acid and α-D-glucose (see below) (Table S1), which was concordant with the phenotype microarray data.

Metabolic reconstructions for each of the four strains were undertaken to analyse whether the phenotypic differences detected could be ascribed to the presence or absence of specific genes. *A. baumannii* D1279779 was unable to utilise 4-hydroxybenzoic acid as a sole source of carbon in either the phenotype microarray (Figure 4) or minimal media (Table S1). Genomic analysis indicated that this strain lacked the *pobA* gene encoding a 4-hydroxybenzoate 3-hydroxylase [79]. Both this strain and *A. baumannii* SDF were the only genomes examined that lacked this gene.

*A. baumannii* ATCC 17978 was the only strain in the test group able to utilise the diastereomers saccharic acid (glucarate) and mucic acid (galactarate) as sole sources of carbon (Figure 4). This was attributable to the presence of a gene cluster involved in the glucarate degradation pathway [80] conserved in some other *A. baumannii* strains, but absent in D1279779, ACICU and AYE (Table 3, Table 4).

Both strains D1279779 and ATCC 17978 showed a positive result for respiration on both D-gluconic acid and α-D-glucose. Our results were concordant with the results of a previous phenotype microarray study [91], which demonstrated the capability of various *Acinetobacter* sp. (including *A. baumannii*) to respire in the presence of both these carbon sources. This is curious, since the majority of *Acinetobacter* species including *A. baumannii* have repeatedly been reported as incapable of utilising D-glucose and D-gluconate as sole carbon sources [92,93]. All four strains encode the Entner-Doudoroff (ED) pathway, an alternative glucose assimilation route that requires the cofactor pyrroloquinoline quinone (PQQ) [94]; a PQQ biosynthetic pathway is evident in three of the strains (but not in ATCC 17978). Growth experiments in both M9 and IF-0 minimal media using D-gluconic acid and α-D-glucose as sole carbon sources, with or without PQQ supplementation, were all negative. The apparent contradiction between the genome, phenotype microarray data and growth assays may indicate these substrates are not assimilated, but rather act as energy donors [95].

A number of the differences observed in carbon and nitrogen utilization between the strains could not be accounted for at the genetic level. This could be due to differences in regulation, membrane transport activity, or the presence of novel uncharacterized catabolic pathways. For instance, strains D1279779 and ATCC 17978 were able to utilize the branched-chain amino acids leucine and isoleucine, while ACICU and AYE were only able to respire weakly on leucine. All of the strains encoded a putative branched-chain amino acid aminotransferase (IlvE) for the

![Figure 4. The catabolic phenome of *Acinetobacter baumannii*. Strengths of carbon utilisation phenotypes of *A. baumannii* strains D1279779, ACICU, AYE and ATCC 17978 were determined using Biolog Phenotype Microarray plates PM1 and PM2. The maximal kinetic curve height was expressed as a greyscale ranging from 101 (light grey) to 320 OmniLog units (black). Phenotypes are arranged from strongest to weakest relative to *A. baumannii* D1279779. Phenotypes ≥101 OmniLog units (white) were considered negative. doi:10.1371/journal.pone.0058628.g004](figures/figure4.png)
reversible transamination of isoleucine, leucine and valine [96], it seems likely the phenotypic differences are due to altered regulation or transport factors. It is possible the residual leucine utilisation in AYE and ACICU is due to a tyrosine aminotransferase (TyrB), which overlaps IlvE in specificity to leucine [97]. In another instance, A. baumannii AYE was found to be unable to utilise proline, ornithine and putrescine as carbon sources (Figure 4) or citrulline, ornithine and putrescine as nitrogen sources (Figure S2). This suggests potential defects in proline and arginine catabolism, but these deficiencies cannot currently be accounted for at the genetic level.

The resistance phenome of A. baumannii D1279779

The osmotolerance (Table S1), pH tolerance (Figure S2) and antimicrobial resistance (Figure 5, Table S1) of the four A. baumannii strains was also examined with Biolog Phenotype MicroArrays. A. baumannii ACICU and ATCC 17978 were found to be more sensitive to acidic pH and were only able to deaminate a limited number of compounds at pH 4.5 (Figure S2). The four A. baumannii strains displayed high intrinsic resistance to many antimicrobial compounds. Respiration in all four strains was observed, at all concentrations, for 94 of the 240 antimicrobials tested (Table S1). No respiration was observed in any strain for five compounds: the antibiotic novobiocin and the heavy metals salts potassium chromate, cadmium chloride, sodium orthovanadate and sodium metavanadate. Differential susceptibility to a further 106 compounds was observed in the four strains (Figure 5, Table S1). Strains D1279779 and ATCC 17978 were noticeably more susceptible to a range of clinically important antibiotics, including the beta-lactams and tetracyclines. The higher levels of resistance of A. baumannii ACICU and AYE towards beta-lactams and sulphonamides is likely due to resistance determinants encoded within their respective AbaR elements [2,9], as well as the carbapenem resistance plasmid pACICU1 [9]. Although, ATCC 17978 was also resistant to sulphonamides, it is likely that this resistance is encoded on a separate genomic island [98]. The strain AYE encodes two TetA tetracycline resistance efflux pumps [2], though in the case of ACICU, there are no characterized tetracycline resistance genes; the observed resistance may be due to the function of other efflux pumps. Resistance to the quinolone nalidixic acid can be accounted for by mutations both in gyrA and parC in strain AYE, and a mutation in gyrD for strain ACICU [8]; neither mutation is present in ATCC 17978 or D1279779. A. baumannii AYE was found to be relatively more resistant to arsenic and rifampicin SV, attributable to the respective presence of an arsenic resistance cluster and a rifampin ADP-ribosyltransferase (arr-2) in its AbaR island [2]. Strains AYE and ATCC 17978 had increased resistance to copper, very likely due to the presence of a copper resistance cluster (copABCDRS) [8], in addition to the copper resistance genes (pcoAB) present in the A. baumannii core genome.

Conclusions

The CA-AB isolate D1279779, while phylogenetically related to the ICII A. baumannii global clonal lineage, phenotypically
resembles ATCC 17978 in terms of carbon and nitrogen utilization, and drug susceptibility profile. Phenotypic testing of nosocomial Acinetobacter baumannii suggests that the narrowing of substrate utilisation capabilities and expansion of drug resistance profiles in both ICI and ICH global clonal lineages has contributed to their success in the nosocomial setting. One genomic analysis of the CA-AB isolate D1279779 reveals the absence of the AbsR island common to nosocomial isolates. D1279779 does however comprise of 24 novel RGPs that encode catabolic functions, polysaccharide biosynthesis and many hypothetical proteins of unknown function. Reports in the literature have suggested that CA-AB is associated with higher mortality rates than nosocomial A. baumannii strains. Whilst there were no obvious virulence-associated genes unique to D1279779, there was however, the apparent loss of several genes associated with virulence, particularly with respect to biofilm formation and eukaryotic cell adhesion. The characteristics of D1279779 may be more representative of an environmental or pre-antibiotic era clinical A. baumannii isolate, and appears quite distinct from the current dominant lineages of nosocomial isolates.

Supporting Information
Figure S1 Synteny of Acinetobacter baumannii. Chromosomal alignments of the A. baumannii D1279779 genome against the ten currently complete A. baumannii genomes were generated using progressive MAUVE [103]. Regions of significant synteny between the strains are shown as coloured blocks and unshared regions are seen as white gaps. (PNG)

Figure S2 Phenotypic analysis of nitrogen utilisation and pH stress tolerance. Strengths of nitrogen utilisation (A) and the pH tolerance phenotypes (B) of A. baumannii strains D1279779, ACICU, AYE and ATCC 17978 were determined using Biolog Phenotype Microarray plates PM3 and PM10, respectively. The maximal kinetic curve height was expressed as a greyscale ranging from 101 OmniLog units (light grey) to 310 and 360 OmniLog units (black) for nitrogen and pH tolerance phenotypes, respectively. Phenotypes are arranged from strongest to weakest relative to A. baumannii D1279779. Phenotypes <115 OmniLog units for nitrogen phenotypes and <101 OmniLog units for pH tolerance phenotypes were considered negative phenotypes and are represented in white. (TIF)

Table S1 Raw phenotype microarray data. The maximal kinetic curve height for all phenotypes obtained from plates PM1-3 (carbon and nitrogen utilisation) and PM9-20 (osmotolerance, pH tolerance and antimicrobial exposure) expressed in OmniLog units. (XLS)

Acknowledgments
We would like to thank Matthias Maiwald for providing A. baumannii D1279779, Alessandra Carattoli for providing A. baumannii ACICU and Patrice Nordmann for providing A. baumannii AYE.

Author Contributions
Conceived and designed the experiments: DNF KAH MHB ITP. Performed the experiments: DNF LDHE KAH BAE SGT BSS. Analyzed the data: DNF LDHE BAE BSS ITP. Wrote the paper: DNF LDHE KAH MHB AYP BCM ITP.

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