COMPARATIVE ANALYSIS OF SURFACE-EXPOSED VIRULENCE FACTORS OF ACINETOBACTER BAUMANNII

Bart A Eijkelkamp1,2, Uwe H Stroeher1, Karl A Hassan3, Ian T Paulsen3 and Melissa H Brown1*

Abstract

Background: Acinetobacter baumannii is a significant hospital pathogen, particularly due to the dissemination of highly multidrug resistant isolates. Genome data have revealed that A. baumannii is highly genetically diverse, which correlates with major variations seen at the phenotypic level. Thus far, comparative genomic studies have been aimed at identifying resistance determinants in A. baumannii. In this study, we extend and expand on these analyses to gain greater insight into the virulence factors across eight A. baumannii strains which are clonally, temporally and geographically distinct, and includes an isolate considered non-pathogenic and a community-acquired A. baumannii.

Results: We have identified a large number of genes in the A. baumannii genomes that are known to play a role in virulence in other pathogens, such as the recently studied proline-alanine-alanine-arginine (PAAR)-repeat domains of the type VI secretion systems. Not surprising, many virulence candidates appear to be part of the A. baumannii core genome of virulent isolates but were often found to be insertionally disrupted in the avirulent A. baumannii strain SDF. Our study also reveals that many known or putative virulence determinants are restricted to specific clonal lineages, which suggests that these virulence determinants may be crucial for the success of these widespread common clones. It has previously been suggested that the high level of intrinsic and adaptive resistance has enabled the widespread presence of A. baumannii in the hospital environment. This appears to have facilitated the expansion of its repertoire of virulence traits, as in general, the nosocomial strains in this study possess more virulence genes compared to the community-acquired isolate.

Conclusions: Major genetic variation in known or putative virulence factors was seen across the eight strains included in this study, suggesting that virulence mechanisms are complex and multifaceted in A. baumannii. Overall, these analyses increase our understanding of A. baumannii pathogenicity and will assist in future studies determining the significance of virulence factors within clonal lineages and/or across the species.

Keywords: Genomics, Virulome, Type VI secretion systems, Membrane

Background

Acinetobacter baumannii is a formidable Gram-negative human pathogen that is predominant in hospitals where it is a common cause of infections in critically ill patients in intensive care units and in particular with using respiratory assistance [1]. Clinical A. baumannii isolates display major phenotypic differences in virulence-associated phenotypes such as, biofilm formation, adherence to human epithelial cells, invasion, motility and cytotoxicity [2-6].

No doubt, adherence of A. baumannii on surfaces of medical devices is critical for its spread within the hospital ward and between patients. A recent study has suggested that equipment such as portable X-ray equipment or wheel chairs can be cross-infected with A. baumannii due to aerolization of the organism, thereby negating the need for direct person-to-person contact [7,8]. Furthermore, colonization of ventilators or catheters can be directly related to the occurrence of pneumonia, urinary tract infections and bacteremia [9,10]. Ventilators and catheters may become reservoirs for pathogens such as A. baumannii as these niches are not necessarily exposed to the antimicrobial agents administered to the patients nor are they accessible during normal hospital cleaning procedures. Certainly, longer hospital stays are likely to induce an increased risk of A.
**Results and discussion**

**Strains selected for comparative analyses**

The genomic data made available over the last decade have significantly advanced our knowledge of *A. baumannii*. However, the number of studies in which virulence traits are examined at a genetic level is limited and a broad-scale comparative analysis of these traits between strains that differ in their virulence potential is warranted. In our study, a diverse collection of *A. baumannii* strains, isolated across different geographic and temporal regions, was examined to comprehensively assess the surface-exposed components of the *A. baumannii* virulome. Two isolates from the international clone (IC) I lineage (AB0057 and 6870155) and two from the IC II lineage (ACICU and W/M99c) were included. To gain greater insight into clonal conservation, the two selected isolates per IC lineage were isolated from different continents (Table 1). The remaining four isolates examined in this study did not group within the defined IC lineages. Three of these are the widely studied strains ATCC 17978, ATCC 19606T and SDF. Additionally, both ATCC 17978 and ATCC 19606T were identified more than 50 years ago, making them appropriate targets to examine temporal differentiation. The *A. baumannii* strain D1279779 was isolated from an outpatient from a remote area in tropical Australia and therefore represents the first fully sequenced community-acquired *A. baumannii* isolate [26], whereas SDF is the only fully sequenced representative of a non-pathogenic isolate.

A genome-wide analysis of the shared gene content was performed which showed that 1560 genes were shared between all eight strains, which can be considered the “core genome”. However, strain SDF has undergone major genomic rearrangements and the remaining seven strains have an additional 503 shared genes, hence, 2063 genes were shared between the virulent strains included in this study. In respect to divergence from the core genome (1560 genes), strain ATCC 17978 was found to possess the highest number of unique genes (n = 819) followed by the SDF isolate (n = 606) (Figure 1A). The direct genome comparison revealed that clonality, examined as described previously [33], correlated with their total shared gene content; isolates within clonal groups showed the highest percentage of shared genes as compared to strains from different clonal groups (Figure 1B; Additional file 1). Strain SDF shared the lowest number of genes with other isolates. However, since SDF has a small genome that has undergone considerable genome reduction (only ~3050 open reading frames) it still shared an average of approximately 71% of its coding content with the other isolates used in this analysis. Strain ATCC 17978 also showed comparatively low numbers of shared genes, potentially resulting from its temporal and/or geographic separation from the other strains. The highest proportion of shared genes was 92% between the two IC I isolates AB0057 and 6870155. Overall, these diverse strains exemplify an excellent representation of *A. baumannii* isolates for tracking the spread of virulence determinants, by including clonal strains commonly isolated worldwide (the IC strains), a community-acquired strain and strains that are geographically and
temporally distant. The avirulent isolate SDF was predominantly included for comparative purposes.

Type I pili
One of the most common protein structures decorating the outer surface of pathogens are the Type I pili, which often play a major role in adherence of Gram-negative pathogens, and are well documented in another member of the gamma-proteobacteria, uropathogenic *Escherichia coli* [34]. Four gene clusters encoding these pili have been identified in *A. baumannii* (Figure 2), which include the functionally characterized *csu*-cluster (A1S_2213-2218) [35-37]. Interestingly, the *csu*-cluster is likely to be non-functional in two strains; in ACICU as a result of an insertion, and in ATCC 17978 due to a single nucleotide polymorphism resulting in truncation of *csuB*, as previously described [3]. At a proteomic level, the P pilus annotated proteins (cluster AB57_2003-2007), CsuC and CsuD, and putative Type III pili (Table 2; A1S_0690-0695), were found to be highly expressed in cells in the pellicle [38] perhaps highlighting their role in this phenotype.

Gene clusters orthologous to A1S_1507-1510 (fimbrial genes) are well conserved across the seven virulent *A. baumannii* strains included in this study (Figure 2). Interestingly, this cluster was found to be down-regulated in strain ATCC 17978 under iron-limitation [39], which has adverse effects on pellicle formation (Eijkelkamp et al., unpublished data). Furthermore, we have recently examined an ATCC 17978 derivative, which carries an insertionally-inactivated copy of the major global regulator H-NS [22]. Increased expression of A1S_1507-1510 and enhanced pellicle formation was noted in this mutant strain [22]. A recent study by Rumbo-Feal et al. examining the expression of genes in biofilm versus planktonic cells in strain ATCC 17978 has shown that A1S_1507 is highly up-regulated in biofilm cells compared to planktonic cells as were the *csu* genes *csuD*, *csuC* and *csuA/B* [40]. Therefore, Type I pili are likely to play a role in *A. baumannii* adherence and biofilm/pellicle formation, however, the exact contribution of these three clusters under different conditions requires further examination.

The role of the A1S_2088-2091 Type I pili cluster in *A. baumannii* virulence has not been established. The

### Table 1 Strain characteristics

| Clonality | Strain   | Site of isolation       | Country of origin | Reference |
|----------|----------|-------------------------|-------------------|-----------|
| IC I     | 687015S  | Sputum                  | Australia         | [3]       |
|          | AB0057   | Blood                   | USA               | [27]      |
| IC II    | WM99c    | Sputum                  | Australia         | [3]       |
|          | ACICU    | Cerebrospinal fluid     | Italy             | [28]      |
| Non-IC   | ATCC 17978 | Meninges              | France            | [29,30]  |
|          | ATCC 19606T | Urine                   | USA               | [31]      |
|          | D1279779 | Blood                   | Australia         | [26]      |
|          | SDF      | Louse                   | France            | [32]      |

---

**Figure 1** Genomic diversity among *Acinetobacter* strains. (A) Partial Venn diagram. Each *A. baumannii* strain is depicted by an oval colored according to IC designation. Numbers presented in overlapping regions of the ovals show the number of genes shared by that group of strains. For example, the core genome size shown in the center, where all ovals overlap, is 1560 ORFs. Numbers in the non-overlapping regions show the number of genes unique to that strain. (B) Phylogenetic tree based on a concatenated alignment of the core housekeeping genes *cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB* and *rpoB* in the strains under investigation. *A. baylyi* ADP1 (GenBank; CR543861) was used as an outgroup and the dotted line used in the ADP1 branch indicates a branch length greater than that shown. Numbers to the right of the tree show the size of the total number of conserved ORFs (the core genome) in the set of strains indicated.
IC II isolates appear to lack this cluster and the genomic region in strain SDF appears to have undergone major rearrangements (Figure 2). Furthermore, Sanger sequencing confirmed sequence length variation in a polymeric tract at the 5′-end of A1S_2091 which has rendered this cluster inactive in ATCC 17978 and ATCC 19606T [3]. The poly-thymine tract found in the 5′-end of A1S_2091 may represent the first phase-variation mechanism of \textit{A. baumannii}. During replication, the probability of ‘slippage’ in polymeric tracts can be relatively high [41], which may result in a change of the A1S_2091 open reading frame (ORF).

**Type IV fimbriae**
The Type IV fimbriae (TFF) are large protein complexes with structural components in both the inner and outer membranes. Whereas Type I pili have been implicated in biofilm formation, cell aggregation and attachment, the TFF have been shown to be involved in motility [23,42] and are likely to play a role in virulence in \textit{A. baumannii}, although, this has not been proven to date. Unlike earlier reports on the absence of \textit{pilX}, \textit{pilV} and \textit{pilT} of the TFF in strain ATCC 17978 (Table S2 in Antunes et al. [43]), we found that strain ATCC 17978 possesses all orthologous genes identified, in for example, strain ACICU. In fact, SDF was found to be the only strain lacking various TFF genes as a result of insertional disruption/deletion events, corroborating the findings by Antunes and colleagues. Notably, the A1S_3166 (\textit{pilE}) ortholog is truncated in strain ATCC 19606T as a result of an adenosine deletion in a position that correlates to 3,648,940 in strain ATCC 17978. Whether this potential nucleotide deletion has phenotypic consequences remains to be examined. Despite the presence of the individual genes required for TFF biosynthesis in most strains, there are significant differences in the

**Table 2 Comparative analysis of surface-presented protein structures**

| Gene/locus-tag | Gene product/function | 6870155 | AB0057 | WM99c | ACICU | ATCC 17978 | ATCC 19606T | D1279779 | SDF |
|----------------|-----------------------|---------|--------|-------|-------|------------|------------|--------|-----|
| A1S_1032       | Autotransporter adhesin | -       | -      | +     | +     | -          | +          | +*     | -   |
| A1S_2696       | Biofilm associated protein | +       | +      | +     | +     | +          | -          | -      | +   |
| AB57_3081      | Biofilm associated protein | +       | +      | +     | +     | -          | -          | +*     | -   |
| A1S_2840       | OmpA                   | +       | +      | +     | +     | +          | -          | -      | -   |
| A1S_0884       | OmpA-like              | +       | +      | +     | +     | +          | +          | +      | +   |
| A1S_1033       | OmpA-like              | -       | -      | +     | +     | +          | +          | -      | -   |
| A1S_1193       | OmpA-like              | +       | +*     | +     | +     | +          | +          | +      | +   |
| A1S_0108       | Pmt                    | +       | +      | +     | +     | +          | +          | +      | -   |
| A1S_0269       | Type II secretion system | +       | +      | +     | +     | +          | +          | +      | +   |
| A1S_0690-0695  | Putative Type III pilis | +       | +      | +     | +     | +          | +          | -      | +   |

*Pseudogenes.
+Gene present.
-Gene absent.
major fimbrial subunit (PilA) between strains. We have previously shown that PilA, is highly variable between strains and that this correlates with differences in their motility characteristics [3].

**Type V secretion systems**
The significance of Type V secretion systems, also called autotransporter proteins, in bacterial pathogenesis has been well documented [44-46]. The autotransporter Ata from *A. baumannii* strain ATCC 17978 has been shown to play an important role in adherence and virulence [47]. Our analyses indicated that this might be the only autotransporter in *A. baumannii*. Homologs of Ata (A1S_1032) were only found in the IC II strains WM99c and ACICU, and in the non-IC strains ATCC 17978, ATCC 19606T and D1279779 (Table 2). However, Ata is most likely non-functional in strain D1279779 as the ORF has been truncated significantly at the 5’-end. Major sequence variation was seen in the central region of this gene; what impact this may have on virulence is unknown. The autotransporter gene appears to be co-transcribed with a gene encoding a putative OmpA-like protein (A1S_1033). We recently showed that these two genes are also regulated by H-NS [22]. H-NS is known to play an important role in adherence and virulence [47]. Our analyses indicated that at least 11 unique insertion events have taken place in the eight *A. baumannii* strains [47]. We have previously shown that this isolate produces a poor biofilm as compared to other *A. baumannii* strains [3]. The exact role Ata plays in virulence and adherence in strains other than ATCC 17978 has yet to be elucidated.

**Type VI secretion systems**
The Type VI secretion systems (T6SSs) are known to be involved in cell invasion and competition amongst bacteria and have been identified in a number of pathogens [48-50]. T6SSs have also been proposed to play a role in Type I pili regulation and inter-bacterial communication [51-53]. Their ubiquitous presence in both pathogenic and non-pathogenic bacteria also shows that these phage-related protein structures are involved in more functions than solely pathogenicity [48,54]. The function of T6SS in *A. baumannii* has been reported in a limited number of studies. An ATCC 17978-derived T6SS mutant was found to be uncompromised in virulence and bacterial competition studies [55]. However, in a second publication examining the role of T6SS in bacterial competition, *Acinetobacter nosocomialis* strain M2 was found to utilize the T6SS for killing of *E. coli* [23,56] potentially giving strains expressing T6SSs a competitive advantage.

The gene cluster encoding the *A. baumannii* T6SS was identified in all strains included in this study, except for the community-acquired isolate D1279779. Furthermore, part of the T6SS cluster in the avirulent strain SDF (ABSDF2238-2242) displayed low levels of sequence similarity with orthologs in the other *A. baumannii* strains (data not shown), which may result in functional differentiation of the T6SS in strain SDF, as has been illustrated in *Pseudomonas aeruginosa* [54,57]. The common *P. aeruginosa* reference strain, PA01, harbors three T6SSs with distinct functions; these being eukaryotic cell invasion and inter-bacterial communication/competition [54,57]. Comparative analysis of the *A. baumannii* T6SS to the functionally defined T6SSs from *P. aeruginosa* did not show higher sequence similarity between particular members, hence, the function of the T6SSs in the strains examined here remains to be determined experimentally.

Three different types of putative effectors of the *A. baumannii* T6SSs have been identified; Hcp (hemolysis co-regulated protein), which is encoded by the highly conserved gene A1S_1296 (co-expressed with the other structural components of the T6SS), and a multitude of Valine-Glycine-Repeat protein G-like (VgrG-like) and Proline-Alanine-Alanine-Arginine (PAAR)-repeat domain proteins. Despite the high levels of homology observed between most T6SS clusters, the genes encoding the VgrG and PAAR-repeat domain proteins were found to be scattered throughout the genomes and only a few of these were found to be conserved between strains (Figure 3). The scattering of vgr-like genes is not unusual and has been documented in other bacteria such as *Vibrio cholerae* and *E. coli* where the vgr-like genes were initially thought to be accessory components of the recombination hot spot family [58,59]. One of the genes annotated as a ‘Vgr-related’ protein encoded by A1S_3364 in strain ATCC 17978 was identified in all eight *Acinetobacter* strains, however, the C-terminal domain is highly variable. Furthermore, a major truncation of the gene encoding this VgrG protein appears to have occurred in strains ATCC 19606T and SDF, which likely renders it inactive. A number of vgrG genes were also identified in close proximity to the genomic position of the T6SS itself (Figure 3), which is not unusual and has been observed in numerous other bacteria [50]. However, the exact location of this gene, represented by A1S_1288 in strain ATCC 17978, differs and homologs can only be found in strains 6870155, AB0057, WM99c and ACICU. Overall, our comparative analyses indicated that at least 11 unique insertion events of vgrG have taken place in the eight *A. baumannii* strains.
included in this study (Figure 3). Most strains harbor three full-length vgrG genes, including D1279779, which does not possess the T6SS itself; ACICU possesses four.

A recent report on PAAR-repeat proteins demonstrated their significance in T6SS-mediated bacterial competition [60]. The PAAR-repeat domain was found to dock onto the terminus of the pilus-like structure formed by VgrG, sharpening the T6SS complex and enabling penetration of host cells. Schneider and co-workers have subdivided the PAAR-repeat proteins into seven different classes, depending on the presence of additional C- and/or N-terminal domains. We identified class 1, 2 and 5 PAAR-repeat proteins in the A. baumannii genome (Figure 3 and Additional file 2), all of which showed highest homology to the pfam05488 type of PAAR-repeat domains (data not shown). The class 1 PAAR-repeat proteins contain no additional domains and the genes encoding these in A. baumannii were predominantly found clustered with the other components of the T6SS system, such as A1S_1306 in strain ATCC 17978. A total of 12 class 2 PAAR-repeat proteins were identified, with strain SDF harboring seven, of which six were identified on the chromosome and one on plasmid p2ABSDF (Additional file 2). The proteins within this class possess two domains, the PAAR-repeat domain positioned N-terminally and in case of the A. baumannii class 2 proteins a C-terminal domain of unknown function. Only strains ATCC 19606T, ACICU and SDF possess a member of the class 5 PAAR-repeat proteins. These proteins are composed of three domains with the PAAR-repeat domain centralized. Unfortunately, none of the N- and C-terminal extensions of this class in A. baumannii encode domains with known functions. The previously characterized PAAR-repeat proteins ACIAD2681, ACIAD0052, ACIAD0051 from Acinetobacter baylyi ADP1 [60] were found to cluster within class 1, 2 and 5, respectively (Additional file 2). There are major differences in the number of PAAR-repeat proteins between the strains in this study, as none were identified in strain D1279779 and SDF was found to possess 10, representing members of three distinct classes. The absence of genes encoding the PAAR-repeat containing proteins in strain D1279779 correlates with the lack of the structural components of the T6SS.

We have recently shown that H-NS regulates expression of the T6SS cluster [22], however, the whole transcriptome analysis also indicated that the genes encoding VgrG-like proteins or PAAR-repeat proteins are not under regulatory control of H-NS. Further work is required to elucidate the function of the different types of VgrG-like proteins and PAAR-repeat proteins in A. baumannii.

Additional membrane-associated protein structures

Protein or toxin secretion systems play an important role in pathogenesis [61] and in order for proteins to be secreted they need to cross both the cytoplasmic and outer membrane of A. baumannii. We identified various genes encoding complex protein structures that could mediate this process (Table 2). Unfortunately, to date the characterization of these systems in A. baumannii has been limited. Screening of a transposon library, revealed that insertions in A1S_0269 abrogated protection during growth in human serum [62]. An insertion in A1S_0269 may have polar effects on the co-transcribed genes A1S_0270 and A1S_0271. The A1S_0269 gene is

---

Figure 3 Genomic positioning of genes encoding components of the Type VI secretion system. The complete genomes (horizontal black lines) have been shown to scale. The black arrows indicate the position of the cluster encoding the type VI secretion system; this cluster is not present in strain D1279779 with the grey arrow pointing to its predicted position. The rectangles indicate vgrG-like genes, their genomic position and the color show orthology (E-value <10^{-30}). No significant similarity was seen between those represented in white. Rectangles without borders represent disrupted vgrG-like genes. The genomic position of genes encoding PAAR-repeat domain proteins has been indicated by the block-arrows. The color indicates to which class the encoded PAAR-repeat domain belongs; class 1 (red), class 2 (black) and class 5 (blue).
predicted to produce the N component of a putative Type II secretion system which has been shown to be non-essential for Type II secretion [63], A1S_0270 encodes part of the inner membrane platform and A1S_0271 encodes the outer membrane secretin/channel. Exactly how protection to human serum is mediated requires further characterization. We found the three components of this putative Type II secretion system to be highly conserved between all A. baumannii strains included in this study (Table 2). However, an insertion sequence has disrupted the upstream region of this cluster in strain SDF.

The role of the outer membrane protein OmpA (A1S_2840) in A. baumannii virulence has been studied intensively [12,64-67] and has been shown to facilitate adherence to eukaryotic cell surfaces and cell invasion [12,65]. Furthermore, OmpA promotes cell death of lung epithelial cells by induction of interleukin-8 and other cytokines [14,68,69]. OmpA was found to be well conserved amongst all eight isolates. Three additional ompA-like genes were identified in the A. baumannii genomes (A1S_0884, A1S_1033 and A1S_1193). Both A1S_0884 and A1S_1193 were identified in all eight strains, however, a frame-shift in AB57_1330 may render this ompA-like gene inactive in strain AB0057 (Table 2). As described above, A1S_1033 appears to be co-transcribed with the autotransporter aty and was identified only in the IC II strains, WM99c and ACICU, and in strains ATCC 17978, ATCC 19606T and D1279779.

The biofilm associated protein (Bap) facilitates adherence in A. baumannii [70-72] and is likely to be involved in virulence as shown in other pathogens [73]. This large protein shows major sequence variation, which has previously been described in detail [70,72]. The bap gene in strain ACICU appears to have been annotated as a number of distinct ORFs and requires sequence confirmation. Furthermore, Loehfelm et al. [71] described the identification of two distinct Bap-like proteins in strain ATCC 17978 and genome sequence analysis indicated that the genes encoding these fragments, A1S_2724 and A1S_2696, may actually have been separated by rearrangement events [71]. A second Bap-like protein encoded by AB57_3081 in A. baumannii strain AB0057 can also be identified in strains 6870155, WM99c, ACICU and ATCC 19606T (Table 2). An insertion sequence leads to truncation of this protein in strain SDF, whereas strains ATCC 17978 and D1279779 do not possess any sequences homologous to AB57_3081. The community acquired isolate D1279779 appears to encode no Bap or Bap-like proteins (Table 2).

The major facilitator superfamily (MFS) transporter Pmt has been shown to play a role in adhesion and biofilm formation [74]. Analysis of pmt expression revealed that it was elevated in cells in the biofilm when compared to their planktonic counterparts. Furthermore, expression of pmt in E. coli DH5α cells increased adherence to abiotic and biotic surfaces clearly indicating that this protein plays a role in adherence and, as such, is likely to function in virulence [74]. The mode of action is thought to involve secretion of extracellular DNA which has previously been reported for A. baumannii [74]. The pmt gene (A1S_0108) was identified in all strains regardless of their virulence potential or their ability to form biofilms (Table 2).

Capsule biosynthesis

The O antigen and/or the capsule have long been recognized as essential virulence factors in numerous bacterial species as mutants that are either rough (contain no O antigen) or acapsular are generally avirulent [75]. By definition, O antigen is linked to the outer core of the lipopolysaccharide (LPS), which constitutes the outer leaflet of the outer membrane in Gram-negative organisms, whereas a capsule is generally considered to be unlinked or linked to the cell wall. There is mounting evidence that A. baumannii produces capsule [76-79], because the waaL gene is lacking within the surface polysaccharide operon. WaaL is required for linking O antigen to the outer core, thus these strains are likely to produce capsule and not O antigen. A waaL-like gene present elsewhere in A. baumannii genomes was thought to be involved in protein glycosylation and not O-antigen linkage [80]. However, Wright et al. [81], has suggested that a second waaL gene present in some strains of A. baumannii may actually be involved in O-antigen linkage as this second gene does not have the domain thought to be required for protein glycosylation [81]. Examination of the strains in our study showed that only the EC II clone strains (ACICU and WM99c) and ATCC 19606T and 6870155 possessed homologs of this second waaL-like gene (Table 3). Comparison of the genome organization of ATCC 17978 encompassing ORFs A1S_0049 to A1S_0066 (Figure 4; Table 3) with the other seven strains used in this study indicates at least six different genetic arrangements of this region, which would imply six different capsule types. This supports the notion that many distinct cluster types may be present across the species [76,77,79]. At the start of this region there are four conserved ORFs (A1S_0049-0052). Adjacent to these highly conserved genes is a genetic region of higher variability, encoding proteins involved in the biosynthesis of specific activated sugar precursors and transferases for each of the capsule types. The last section of this locus contains a second conserved region of five ORFs (A1S_0062 to A1S_0066).

The enzymes involved in the biosynthesis of the various precursors and activated sugars closely match the requirements for the surface polysaccharide structure determined for A. baumannii [82-84]. A recent
publication by Kenyon and Hall (2013) has identified nine genetically distinct capsule loci in *A. baumannii* designated KL1-KL9 and includes a more detailed analysis of the various regions within these loci beyond what is discussed here. The study by Hu *et al.* [79] has extended this to 25 well defined serovars and followed from an earlier study examining 152 *A. baumannii* isolates using polyclonal antisera which revealed that there are possibly over 20 distinct surface polysaccharide types in this organism [85]. Thus, there appears to be significant variation in both the genetic content and arrangement of the proposed capsule loci. Furthermore, examination by Hu *et al.* [79] of the capsule operon of other fully or partially sequenced *A. baumannii* genomes found significant differences in the *wzy* (polymerase) and *wzx* (translocase) genes indicating that there are significantly more serovars than the 25 well described types [79]. In *Acinetobacter* there are currently 77 distinct polysaccharide gene clusters not all of which have to date been identified in *A. baumannii*. No doubt further sequencing may reveal details of these other serovars.

**Lipid A modifications**

The lipid A fraction of LPS is considered a major virulence factor of Gram-negative bacteria. In *A. baumannii* lipid A has predominantly been studied for its role in resistance to the polymyxin antimicrobial peptides [86-90]. Interestingly, the modifications of lipid A that are associated with polymyxin resistance may negatively affect the virulence potential, a topic currently under debate [91,92]. Lipid A modification occurs as a result of up-regulation of *pmrC* (A1S_2752), a gene encoding a lipid A phosphoethanolamine transferase. We found several other genes encoding lipid A phosphoethanolamine transferases in the genomes examined in this study, which may affect antigenicity of this membrane component and/or polymyxin susceptibility. Whereas *pmrC* is highly conserved across all genomes with the exception of strain SDF, the additional phosphoethanolamine transferases were found to be most often associated with horizontally-acquired regions. For example, ACICU_01072 was found in a large phage-related cluster in the IC II (ACICU and WM99c) strains only. The community-acquired strain D1279779 was found to possess a second distinct phosphoethanolamine transferase (Table 3).

**Other carbohydrate-based surface decorations**

The *A. baumannii* strains included in this study appear to possess two clusters encoding proteins required for biosynthesis of poly-β-1-6-N-acetylglucosamine (PNAG),

![Figure 4](image_url)

*Figure 4 Genomic region involved in production and secretion of capsule.* Genomic organization of the region involved in capsule biosynthesis. Genes have been drawn to scale. The arrows represent ORFs and depict the direction of transcription; capsule biosynthesis genes (black), adjacent homologous genes (grey) and transposases (red). The blue shading indicates a high level of homology between genes from different strains (E-value <10^-25).
a polysaccharide that is critical for biofilm formation. Only one of the gene clusters (A1S_3792, A1S_2160-2162) has been functionally examined [25]. As observed with the characterized cluster, the second cluster (A1S_0938-0940) is highly conserved across most strains; both clusters have been removed by insertion-deletion events in strain SDF (Table 3).

A large gene cluster (A1S_0112-0119) believed to be responsible for the production of a biosurfactant, most likely in the form of lipopeptides, has been shown to play a role in surface motility [42]. Intriguingly, expression of this cluster is affected by differing levels of quorum-sensing signals [42], the global regulatory protein H-NS [22] and cyclic-AMP (Giles et al., unpublished data). This cluster and the DNA-binding site for the quorum-sensing regulator AbaR was identified in all seven virulent strains, however, there is major sequence variation in other parts of the upstream region (data not shown), which could result in differential repression by H-NS.

Conclusions
In this study we sought to examine the surface-exposed virulence factors of the human pathogen A. baumannii. We described for the first time a range of novel prospective virulence candidates, including a fourth Type I pilus cluster (A1S_2088-2091), the VgrG-like proteins, the PAAR-repeat domain proteins, Bap-like proteins and various OmpA-like proteins. Furthermore, we identified additional phosphoethanolamine transferases and a gene cluster putatively involved in the production of PNAG (A1S_0938-0940). All of these candidates make excellent targets for experimental examination.

Our genome comparison data show that many of the highly conserved A. baumannii virulence genes have been insertionally-disrupted or mutated in strain SDF, providing insight into its non-pathogenic phenotype. As reported previously by our group when studying carbon and nitrogen utilization and drug resistance [26], the community-acquired A. baumannii isolate D1279779 was found to be quite distinct from its nosocomial counterpart. Accordingly, in this study we found that strain D1279779 possessed significantly fewer virulence genes. Thus far, the link between the virulence potential of community-acquired A. baumannii strains, and the virulence and drug resistance genes has not been fully explored. Multiple horizontally-acquired genomic regions found in the common IC clone strains were also identified in strain D1279779, such as the OmpA-like protein encoded by A1S_1033. This indicates that although geographically isolated, community-acquired isolates appear to be capable of acquiring novel virulence genes. Alternatively, these genes could have been acquired by lateral gene transfer by a common ancestor before the split of the strains. In conclusion, our study shows that the common nosocomial clones have a broad and diverse repertoire of virulence genes, which in combination with their extensive range of resistance genes provides the genotype fundamental to the success of this species.

Methods

Genome mining and comparative analyses
Identification of orthologous genes/proteins across the A. baumannii genomes was performed using blastp searches (E-value <10e-30) in either NCBI [93] or RAST [94]. All eight genomes (GenBank accession numbers can be found in Additional file 1) were aligned using Mauve [95], which was subsequently used for determining genetic positioning and identification of insertion events. The genomic alignment from Mauve was also utilized as a template for generating Figures 2 and 4.

The putative proteomes of the eight strains included in this study were compared using blastp 2.2.25+. Putative orthologs were assigned as reciprocal best hits with an E-value <10e-5 and length match greater than 75%.

Phylogenetic analysis of PAAR-repeat domains
The proteins containing PAAR-repeat domains were aligned using ClustalOmega [96]. Only the PAAR-repeat domains were included for generation of the phylogenetic tree using the Neighbor-Joining method (100 bootstrap analysis replicates) [97]. The tree was visualized in CLC Sequence Viewer 6 (CLC bio, Cambridge, MA, USA).

Additional files

Additional file 1: Pairwise gene conservation. The table shows the percentage of genes shared between strains, using a pairwise comparison. The comparison between the IC I isolates has been highlighted in green and between the IC II isolates in red. Also listed are the GenBank accession numbers.

Additional file 2: Phylogenetic analysis of the PAAR-repeat domain containing proteins. The extension sequences of the class 2 and 5 PAAR-repeat proteins in this analysis were excluded to compare the PAAR-repeat domains only, i.e. based on the class 1 PAAR-repeat proteins. The A. baylyi APD1 PAAR-repeat proteins have been included for comparative purposes. The proteins originate from the following strains: _WM99c, WM99c; A1S_; ATCC 17978; HMPREF00210; ATCC 19605; ACICU, ADP1; ACICU, ACOU; ABS7, AB0057, ABDF, SDF, and _6870155, 6870155. The different colors indicate the class to which the highlighted clade belongs, class 1, 2 or 5 as described previously [60].

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
BAE, UHS, KAH, ITP and MHB designed the research project. BAE, UHS and KAH carried out the experiments and BAE, UHS, KAH, ITP and MHB wrote the manuscript. All authors have read and approved the final manuscript.
Acknowledgements

We would like to thank Liam Elbourne for assistance with the assembly of the genome sequence of Acinetobacter baumannii strain 6870155. This work was supported by Project Grant 535053 from the National Health and Medical Research Council, Australia.

Author details
1School of Biological Sciences, Flinders University, Adelaide, Australia.
2Research Centre for Infectious Diseases, School of Molecular and Biomedical Science, University of Adelaide, Adelaide, Australia.
3Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, Australia.

Received: 18 July 2014 Accepted: 14 November 2014 Published: 25 November 2014

References

1. Falagas ME, Karveli EA, Siempkos II, Vardaxis KZ. Acinetobacter infections: a growing threat for critically ill patients. Epidemic Infect 2008, 136(8):1009–1019.
2. McQuade CN, Actis LA. Acinetobacter baumannii biofilms: variations among strains and correlations with other cell properties. J Microbiol 2011, 49(2):423–450.
3. Eijkelkamp BA, Stroeher UH, Hassan KA, Papadimitrious MS, Paulsen IT, Brown MH. Adherence and motility characteristics of Acinetobacter baumannii isolates. FEMS Microbiol Lett 2011, 323:44–51.
4. de Breij A, Dijkshoorn L, Lagendijk E, van der Meer J, Koster A, Bloemberg G. OmpA protein plays a role in biofilm formation on abiotic surfaces and in multiresistant clinical isolates of Acinetobacter baumannii causing urinary tract infection. J Clin Microbiol 2006, 10(3):346–351.
5. Lee JC, Koerten H, van den Broek P, Beekhuis H, Wolterbeek R, Brown MH. Adherence of Acinetobacter baumannii strain 6870155. This work was supported by Project Grant 535053 from the National Health and Medical Research Council, Australia.
6. Martí S, Rodríguez-Bano J, Catel-Ferreira M, Jouenne T, Vila J, Seifert H, Dé E. Twitching motility contributes to the role of pili in corneal infection caused by Pseudomonas aeruginosa. Infect Immun 2003, 71(5):389–393.
7. Eijkelkamp BA, Stroeher UH, Hassan KA, Elbourne LD, Paulsen IT, Brown MH. H-NS plays a role in expression of Acinetobacter baumannii virulence features. Infect Immun 2013, 81(7):2574–2583.
8. Harding CM, Tracy EN, Carruthers MD, Rather PN, Acts LA, Munson RS Jr. Acinetobacter baumannii strain M2 produces type IV pili which play a role in natural transformation and twitching motility but not surface-associated motility. MBio 2013, 4(3):e00660–13.
9. Bahar O, Goffer T, Burdman S. The role of twitching motility in biofilm formation at the solid-liquid and air-liquid interfaces by Acinetobacter baumannii. BMC Res Notes 2011, 4(5):366.
10. Raad I, Reitzel R, Jiang Y, Chemaly RF, Dvorak T, Hachem R. Acknowledgements
11. Raad I, Reitzel R, Jiang Y, Chemaly RF, Dvorak T, Hachem R. The role of twitching motility in biofilm formation on abiotic surfaces and in multiresistant clinical isolates of Acinetobacter baumannii causing urinary tract infection. J Clin Microbiol 2006, 10(3):346–351.
12. Eijkelkamp BA, Stroeher UH, Hassan KA, Elbourne LD, Paulsen IT, Brown MH. H-NS plays a role in expression of Acinetobacter baumannii virulence features. Infect Immun 2013, 81(7):2574–2583.
13. Adams MD, Goglin K, Molyneaux N, Hujer JM, Martin KM, Russo T, Campagnari AA, Hujer AM, Bonomo RA, Gill SR. Comparative genome sequence analysis of multidrug-resistant Acinetobacter baumannii. J Clin Microbiol 2009, 47(11):3953–3963.
14. Farrugia DN, Elbourne LD, Hassan KA, Eijkelkamp BA, Tetu SG, Brown MH, Shah BS, Peleg AY, Abbatt BC, Paulsen IT. The complete genome and phenotype of a community-acquired Acinetobacter baumannii. PLoS One 2013, 8(3):e56826.
15. Adams MD, Goglin K, Molyneaux N, Hujer JM, Martin KM, Russo T, Campagnari AA, Hujer AM, Bonomo RA, Gill SR. Comparative genome sequence analysis of multidrug-resistant Acinetobacter baumannii. J Clin Microbiol 2009, 47(11):3953–3963.
16. Garcia LB: A new dimension in transmission. J Hosp Infect 2013, 83(3):191–198.
17. Shah BS, Peleg AY, Mabbutt BC, Paulsen IT. The role of twitching motility in biofilm formation on abiotic surfaces and in multiresistant clinical isolates of Acinetobacter baumannii causing urinary tract infection. J Clin Microbiol 2006, 10(3):346–351.
18. Alarcon I, Evans DJ, Fleiszig SM. The role of twitching motility in Pseudomonas aeruginosa exit from and translocation of corneal epithelial cells. Invest Ophthalmol Vis Sci 2009, 50(5):2237–2244.
19. Bähler J, Goffer T, Burdman S. Type IV Pili are required for virulence, twitching motility, and biofilm formation of Acidovorax avenae subsp. Citrulli. Mol Plant Microbe Interact 2009, 22(8):909–920.
20. Han X, Kenann RM, Davies JK, Reddick LA, Dhungyel OP, Whittington RJ, Turnbull L, Whitchurch CB, Rood J. Twitching motility is essential for virulence in Dichelobacter nodosus. J Bacteriol 2008, 190(9):3323–3335.
21. Zolfaghar I, Evans DJ, Fleiszig SM: Twitching motility contributes to the role of pili in corneal infection caused by Pseudomonas aeruginosa. Infect Immun 2003, 71(5):389–393.
22. Eijkelkamp BA, Stroeher UH, Hassan KA, Elbourne LD, Paulsen IT, Brown MH. H-NS plays a role in expression of Acinetobacter baumannii virulence features. Infect Immun 2013, 81(7):2574–2583.
23. Harding CM, Tracy EN, Carruthers MD, Rather PN, Acts LA, Munson RS Jr. Acinetobacter baumannii strain M2 produces type IV pili which play a role in natural transformation and twitching motility but not surface-associated motility. MBio 2013, 4(3):e00660–13.
24. Adams MD, Goglin K, Molyneaux N, Hujer JM, Martin KM, Russo T, Campagnari AA, Hujer AM, Bonomo RA, Gill SR. Comparative genome sequence analysis of multidrug-resistant Acinetobacter baumannii. J Clin Microbiol 2009, 47(11):3953–3963.
25. Farrugia DN, Elbourne LD, Hassan KA, Eijkelkamp BA, Tetu SG, Brown MH, Shah BS, Peleg AY, Abbatt BC, Paulsen IT. The complete genome and phenotype of a community-acquired Acinetobacter baumannii. PLoS One 2013, 8(3):e56826.
26. Adams MD, Goglin K, Molyneaux N, Hujer JM, Martin KM, Russo T, Campagnari AA, Hujer AM, Bonomo RA, Gill SR. Comparative genome sequence analysis of multidrug-resistant Acinetobacter baumannii. J Clin Microbiol 2009, 47(11):3953–3963.
27. Adams MD, Goglin K, Molyneaux N, Hujer JM, Martin KM, Russo T, Campagnari AA, Hujer AM, Bonomo RA, Gill SR. Comparative genome sequence analysis of multidrug-resistant Acinetobacter baumannii. J Clin Microbiol 2009, 47(11):3953–3963.
involvement of a novel chaperone-usher pilus assembly system.

Microbiology 2003, 149(12):3473–3484.

37. Tomaras AP, Flagler MJ, Dorsey CW, Gaddy JA, Actis LA: Characterization of a two-component regulatory system from Acinetobacter baumannii that controls biofilm formation and cellular morphology. Microbiology 2008, 154(Pt 11):3299–3305.

38. Martín S, Nadchalane Y, Alexandre S, Coquet L, Vila J, Jouenne T, De E: Growth of Acinetobacter baumannii in pellicle enhanced the expression of potential virulence factors. PLoS One 2011, 6(10):e26030.

39. Eijkelkamp BA, Hassan KA, Paulsen IT, Brown MH: Investigation of the human pathogen Acinetobacter baumannii under iron limiting conditions. BMC Genomics 2011, 12(1):126.

40. Rumbolo-Sal G, Gómez MJ, Gayoso C, Álvarez-Fraga L, Cabral NP, Arsanay AM, Rodriguez-Epuleta N, Fullaondo A, Valle J, Tomás M, Bou G, Poza M: Whole transcriptome analysis of Acinetobacter baumannii assessed by RNA-sequencing reveals different mRNAs expression profiles in biofilm compared to planktonic cells. PLoS One 2013, 8(8):e72968.

41. Orsi RH, Bowden BM, Wiedmann M: Homopolymeric tracts represent a general regulatory mechanism in prokaryotes. BMC Genomics 2010, 11:102.

42. Clemmer KM, Bonomo RA, Rather PN: Genetic analysis of surface motility in Acinetobacter baumannii. Microbiology 2011, 157(9):2534–2544.

43. Antunes LC, Imperi F, Carattoli A, Visca P: Deciphering the multifactorial nature of Acinetobacter baumannii pathogenicity. PLoS One 2011, 6(8):e22674.

44. Allippop S, Beloin C, Ulett GC, Valle J, Totsika M, Sherlock O, Ghigo JM, Schermbr MA: Molecular characterization of UpaB and UpaC, two new autotransporter proteins of uropathogenic Escherichia coli CFT073. Infect Immun 2012, 80(1):321–332.

45. Henderson IR, Navarro-García F, Desvaux M, Fernandez RC, Alsa/Aldean D: Type V protein secretion pathway: the autotransporter story. Microbiol Mol Biol Rev 2004, 68(3):692–744.

46. Lee IC, Grin I, Linke D: Type V secretion: mechanism(s) of autotransport through the bacterial outer membrane. Philos Trans R Soc Lond B Biol Sci 2012, 367(1592):1088–1101.

47. Bentancor LV, Camacho-Peiro A, Bocur-Guzel C, Pier GB, Maina-Litran T: Identification of Ata, a multifunctional trimeric autotransporter of Acinetobacter baumannii. J Bacteriol 2012, 194(13):3950–3960.

48. Cascales E, Cambilau C: Structural biology of type VI secretion systems. Philos Trans R Soc Lond B Biol Sci 2012, 367(1592):1102–1111.

49. Mougous JD, Cuff ME, Raunser S, Chen A, Goodman AL, Joachimik G, Ordóñez CI, Lory S, Walz T, Joachimik M, Mekalanos JJ: A virulence locus of Pseudomonas aeruginosa encodes a protein secretion apparatus. Science 2006, 312(5779):1526–1530.

50. Filouks A, Hachani A, Bieles S: The bacterial type VI secretion machine: yet another player for protein transport across membranes. Microbiology 2008, 154(Pt 6):1570–1583.

51. de Pace F, Nakazato G, Pacheco A, de Paiva JB, Sperando V, da Silveira WD: The type VI secretion system plays a role in type I fimbria expression and pathogenesis of an avian pathogenic Escherichia coli strain. Infect Immun 2010, 78(12):4990–4998.

52. Schwarz S, Hood RD, Mougous JD: What is type VI secretion doing in all those bugs? Trends Microbiol 2010, 18(12):537–537.

53. Schwarz S, West TE, Boyer F, Chiang WC, Carl MA, Hood RD, Rohmer L, Tolker-Nielsen T, Skretsk RV, Mougous JD: Burkholderia type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions. PLoS Pathog 2010, 6(11):e1001068.

54. Jani AJ, Cotter PA: Type VI secretion: not just for pathogenesis anymore. Cell Host Microbe 2010, 8(1):2–6.

55. Weber BS, Miyata ST, Iwashkiw JA, Mortensen BL, Skaar EP, Mougous JD: FM: Genomic and functional analysis of the Type VI secretion system in Acinetobacter. PLoS One 2013, 8(11):e75142.

56. Caruthers MD, Harding CM, Baker BD, Bonomo RA, Hujer KM, Rather PN, Munson RS Jr: Draft genome sequence of the clinical isolate Acinetobacter nosocomialis AIIMS 7 and its closest relative. J Bacteriol 2014, 206(4):1072–1072.

57. Li H, Hua KC, Sauberan SL, Russo TA, Beanan JM, Olson R, Loehfelm TW, Cox AD, Goodlett DR, Mougous JD: Deciphering the multifactorial nature of Acinetobacter baumannii. Glycobiology 2012, 22(12):1800–1809.

58. Sahu PK, Iyer PS, Oak AM, Pasdeki K, Chopade BA: Characterization of eDNA from the clinical strain Acinetobacter baumannii NIH-7 and its role in biofilm formation. ScientificWorldJournal 2012, 2012:973435.

59. Luke NR, Sauberan SL, Russo TA, Beanan JM, Olson R, Loehfelm TW, Cox AD, St Michael F, Vinogradov EV, Campagnari AA: Identification and characterization of a glycosyltransferase involved in Acinetobacter baumannii lipopolysaccharide core biosynthesis. Infect Immun 2010, 78(5):2017–2023.

60. Kenyon JJ, Hall RM: Variation in the complex carbohydrate biosynthesis loci of Acinetobacter baumannii genomes. PLoS One 2013, 8(4):e62160.

61. Kenyon JJ, Marzahl AA, Hall RW, De Castro C: Structure of the K2 capsule associated with the KL2 gene cluster of Acinetobacter baumannii. Glycobiology 2014, 24(5):554–563.

62. Kenyon JJ, Holt KE, Pickard D, Dougan G, Hall RW: Insertions in the OCL1 locus of Acinetobacter baumannii lead to shortened lipooligosaccharides. Rsm Res Microbiol 2014, 165:472–475.
sequencing, and development of a molecular serotyping scheme. PLoS One 2013, 8(7):e70329.
80. Schulz BL, Jen FE, Power PM, Jones CE, Fox KL, Ku SC, Blanchfield JT, Jennings MP: Identification of bacterial protein O-
oligosaccharyltransferases and their glycoprotein substrates. PLoS One 2013, 8(3):e52758.
81. Wright MS, Haft DH, Hanks DM, Perez F, Hujer KM, Bajaksouzian S, Benard MF, Jacobs MR, Bonomo RA, Adams MD: New insights into dissemination and variation of the health care-associated pathogen Acinetobacter baumannii from genomic analysis. MBio 2014, 5(1):e00963-00913.
82. MacLean LL, Perry MB, Chen W, Vinogradov E: The structure of the polysaccharide O-chain of the LPS from Acinetobacter baumannii strain MG1 and SMAL. Carbohydr Res 2011, 346(7):973–977.
83. Vinogradov EV, Brade L, Brade H, Holst O: Structural and serological characterisation of the O-antigenic polysaccharide of the lipopolysaccharide from Acinetobacter baumannii strain 24. Carbohydr Res 2003, 338(23):2751–2756.
84. Traub WH: Acinetobacter baumannii serotyping for delineation of outbreaks of nosocomial cross-infection. J Clin Microbiol 1989, 27(12):2713–2716.
85. Becero A, Liobet E, Aranda J, Bengoechea JA, Doumith M, Horsey M, Dhani H, Chart H, Bou G, Livermore DM, Woodford N: Phosphoethanolamine modification of lipid A in colistin-resistant variants of Acinetobacter baumannii mediated by the pmrAB two-component regulatory system. Antimicrob Agents Chemother 2011, 55(7):3370–3379.
86. Arroyo LA, Herrera CM, Fernandez L, Hanks JV, Trent MS, Hancock RE: The pmrCAB operon mediates polymyxin resistance in Acinetobacter baumannii ATCC 17978 and clinical isolates through phosphoethanolamine modification of lipid A. Antimicrob Agents Chemother 2011, 55(8):3743–3751.
87. Henny R, Vithanage N, Harrison P, Seemann T, Coutts S, Moffatt JH, Nation RL, Li J, Harper M, Adler B, Boyce JD: Colistin-resistant, lipopolysaccharide-deficient Acinetobacter baumannii responds to lipopolysaccharide loss through increased expression of genes involved in the synthesis and transport of lipopolysaccharides, phospholipids, and poly-B-1,6-N-acetylglucosamine. Antimicrob Agents Chemother 2012, 56(1):59–69.
88. Moffatt JH, Harper M, Adler B, Nation RL, Li J, Boyce JD: Insertion sequence ISAba11 is involved in colistin resistance and loss of lipopolysaccharide in Acinetobacter baumannii. Antimicrob Agents Chemother 2011, 55(8):3022–3024.
89. Adams MD, Nickel GC, Bajaksouzian S, Lavender H, Murthy AR, Jacobs MR, Bonomo RA: Resistance to colistin in Acinetobacter baumannii associated with mutations in the PmrAB two-component system. Antimicrob Agents Chemother 2009, 53(9):3628–3634.
90. Rolain JM, Roch A, Castanier M, Papazian L, Raoult D: Acinetobacter baumannii resistant to colistin with impaired virulence: a case report from France. J Infect Dis 2011, 204(7):1146–1147.
91. López-Rojas R, Dominguez-Herrera J, McConnell MJ, Docobo-Peréz F, Smani Y, Fernández-Roeyes M, Rivat J: Impaired virulence and in vivo fitness of colistin-resistant Acinetobacter baumannii. J Infect Dis 2011, 203(4):545–548.
92. Aerts RK, Bartels D, Best AA, Delongh M, Ditz T, Edwards RA, Formsmas K, Gerdes S, Glass EM, Kubel M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Pazczi T, Parello B, Pusich GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O: The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008, 9:75.
93. Darling AC, Mau B, Blattner FR, Perna NT: Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 2004, 14(7):1394–1403.
94. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG: Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 2011, 7:539.
95. Saitou N, Nei M: The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987, 4(4):406–425.