Variation in the OC Locus of *Acinetobacter baumannii* Genomes Predicts Extensive Structural Diversity in the Lipooligosaccharide

**Johanna J. Kenyon, Steven J. Nigro, Ruth M. Hall**

School of Molecular Bioscience, The University of Sydney, Sydney, NSW, Australia

**Abstract**

Lipooligosaccharide (LOS) is a complex surface structure that is linked to many pathogenic properties of *Acinetobacter baumannii*. In *A. baumannii*, the genes responsible for the synthesis of the outer core (OC) component of the LOS are located between *ivdE* and *aspS*. The content of the OC locus is usually variable within a species, and examination of 6 complete and 227 draft *A. baumannii* genome sequences available in GenBank non-redundant and Whole Genome Shotgun databases revealed nine distinct new types, OCL4-OCL12, in addition to the three known ones. The twelve gene clusters fell into two distinct groups, designated Group A and Group B, based on similarities in the genes present. OCL6 (Group B) was unique in that it included genes for the synthesis of L-Rhamnose. Genetic exchange of the different configurations between strains has occurred as some OC forms were found in different sequence types (STs). OCL1 (Group A) was the most widely distributed being present in 18 STs, and OCL6 was found in 16 STs. Variation within clones was also observed, with more than one OC locus type found in the two globally disseminated clones, GC1 and GC2, that include the majority of multiply antibiotic resistant isolates. OCL1 was the most abundant gene cluster in both GC1 and GC2 genomes but GC1 isolates also carried OCL2, OCL3 or OCL5, and OCL3 was also present in GC2. As replacement of the OC locus in the major global clones indicates the presence of sub-lineages, a PCR typing scheme was developed to rapidly distinguish Group A and Group B types, and to distinguish the specific forms found in GC1 and GC2 isolates.

**Introduction**

Lipooligosaccharide (LOS) is a lipid-carbohydrate surface structure that is exclusive to the outer membrane of Gram-negative bacteria. Historically the LOS was believed to be essential for outer-membrane stability and survival [1]. However in *Acinetobacter baumannii*, strains lacking LOS have been isolated [2]. None the less, the LOS of *A. baumannii* has been shown to play crucial roles in cell motility [3], surface adhesion [4], resistance to opsonophagocytic killing [5] and to antimicrobial peptides in human serum [6], as well as in the stimulation of the proinflammatory immune response [2,7]. Modifications of the LOS or its complete loss have also been shown to result in resistance to polymyxin antibiotics such as colistin [2,8–10].

LOS is composed of distinct constituents: the lipid A, which anchors the complex in the outer-leaflet of the outer membrane, and an oligosaccharide. The oligosaccharide consists of an inner core, which is generally conserved in a species, and an outer core (OC) that exhibits variation in the carbohydrate residues it includes and the linkages between them [11]. In Gram-negative bacteria, the genes required to construct the outer core are clustered. In some bacteria, LOS structures can be decorated with a further long polysaccharide (O-antigen) to generate lipopolysaccharide (LPS). However, recent evidence indicates that *A. baumannii* strains form only LOS and a capsule [12–17].

The *A. baumannii* chromosome has two clusters of genes for polysaccharide synthesis [12,18], and one of them clearly represents the K locus for capsule synthesis and export [13,15,17,19]. The second locus, located between *ivdE* and *aspS*, should thus be for the synthesis of the OC component of the LOS. One of only two LOS structures that have been resolved for *A. baumannii*, shown as OC1 in Figure 1, was found in two different isolates, ATCC 19606 [20] and SMAL [21]. We previously found that the draft genome of ATCC 19606 [GenBank accession NZ_GG704577] includes OCL1, and there was a strong correlation between the gene content of OCL1 and the OC1 structure [12]. Recently, the presence of insertion sequences (IS) in three different OCL1 genes was shown to reduce the size of the LOS [16], confirming the role of the OC locus in directing the synthesis of the OC.

Variation in the OC locus is often seen in Gram-negative species, and three variants, designated OCL1, OCL2, and OCL3, were initially identified in *A. baumannii* genomes [12]. These gene clusters (Figure 2A) were between 11 and 12 kb in length and contained 9 genes, mainly ones that encode the glycosyltransferases that catalyze the linkage of sugars in the OC. The differences between these three forms are in only a small portion consisting of 2–3 genes adjacent to *aspS*. In the previous study, seven of the isolates examined belonged to the two globally disseminated clonal
groups, global clone 1 (GC1) and global clone 2 (GC2). These clones account for the majority of extensively antibiotic resistant Acinetobacter baumannii clinical isolates [22–24]. OCL1 was seen in 2 of the 3 GC1 isolates, as well as in all 5 of the GC2 isolates examined. However, the third GC1 isolate carried OCL3 suggesting that the gene cluster at the OC locus can be replaced within this clone [12]. More recently, we found OCL1 in 61 Australian GC2 isolates, but 21 Australian GC1 isolates carried either OCL1 (17), OCL3 (3), or a new type, OCL5 (1), that is not related to the other 3 forms [16]. Annotated versions from these sequences can be found in GenBank accessions FJ172370, JN968483, KF130871 and JN247441 for the OCL1 region, and in GenBank accessions KC118540 and HM590877 for OCL3 and OCL5, respectively.

The OC gene cluster responsible for production of the second resolved OC structure that includes several L-Rhamnose residues [25] has not been identified, indicating that further distinct OC gene clusters remain to be discovered. Acinetobacter baumannii is a pathogen of worldwide concern due to the high prevalence of multiply antibiotic resistant isolates causing nosocomial infections, and the present study aimed to explore the full extent of diversity at the OC locus in this important species. 227 draft genomes in the Whole Genome Shotgun (WGS) database and 6 more complete genomes available in GenBank were used to identify new OC locus configurations. In the remaining 12 GC2 genomes, the OC locus was interrupted by an insertion sequence (IS), and the final distribution of OCL1 and OCL3 in GC2 is shown in Table 1. The IS was identified by matching the sequence at the ends of the appropriate IS insertion mutants [16].

As sequencing efforts have focused on the multiply antibiotic resistant isolates belonging to the global clones, the distribution of OC locus forms in these groups was examined first. Four of the additional complete genomes [GenBank accession numbers CP003856, CP003846, CP003849, and AP01357] were from GC2 isolates and these all carried OCL1. Initially, the GC1 and GC2 draft genomes were searched for the presence of the characterized OCL1, OCL2 and OCL3 gene clusters. In all of the GC1 genomes and in 88 of the GC2 genomes, the OC locus was in a single contig or in 2 contigs that could be directly abutted. Most of the GC1 genomes carried OCL1 (9) or OCL3 (14), which had been seen previously. However, OCL2 was also found in 7 isolates, and the new OCL5 form was found in one ST19 isolate, OIFC074 [GenBank accession AMDE01000046] (Table 1). OCL5 is described in detail below. The GC2 chromosomes carried OCL1 (73) or OCL3 (15), indicating that the gene cluster in the OC locus has also been replaced at least once in this clone.

In the remaining 12 GC2 genomes, the OC locus was interrupted by an insertion sequence (IS), and the final distribution of OCL1 and OCL3 in GC2 is shown in Table 1. The IS was identified by matching the sequence at the ends of the appropriate contigs to sequences available in ISFinder. In 2 isolates carrying OCL3 [GenBank accessions AMSX01000028 and AMHK01000047], ISaba13 interrupted gtrOC10 at the same position. The locations of IS in the 10 isolates with OCL1 is shown in Figure 2B with the identity of the IS and the number of isolates with a specific insertion indicated below. In those cases where a gene is interrupted, it would be expected that the OC structure will be altered as a consequence, as has been reported recently for other IS insertion mutants [16].

### Distribution of OCL1, OCL2 and OCL3 in other Acinetobacter baumannii genomes

The remaining complete genomes [GenBank accession numbers NC_021733 and AERZ01000079] both included OCL2, and in one of them, NC_021733, an ISaba20 element interrupted the weeB gene. Eight draft genomes were from ST3 isolates (Table S3) and all included OCL1. The remaining 96 draft genomes were screened for the presence of OCL1, OCL2 and OCL3. 35 isolates belonging to 15 different STs included OCL1, indicating that OCL1 is very widely distributed (Table 1). Two isolates from ST10 carried OCL2, and a single ST136 isolate carried OCL3.

### Results

**Multi-Locus Sequence Typing (MLST)**

The sequence type (ST) was determined for all Acinetobacter baumannii isolates from the sequence data, using the Institut Pasteur MLST scheme (see Methods). The global clones, GC1 and GC2, correspond to sequence types ST1 and ST2 in this scheme, and single locus variants (SLV) were also classified as clone members. Among the draft genomes, 31 were found to be from GC1 isolates (27 ST1, 2 ST19 and 2 ST81) and 100 from GC2 isolates (99 ST2 and 1 ST47). A list of GC1 and GC2 isolates with draft genomes, together with accession numbers, is compiled in Table S1 and Table S2, respectively. A further 51 different STs were found, including ST3 (corresponding to European clone III; Table S3) and 19 new profiles. The genomes examined thus represent a broad range of Acinetobacter baumannii strains suitable for detection of novel OC locus configurations.

**OC locus distribution in GC1 and GC2**

As sequencing efforts have focused on the multiply antibiotic resistant isolates belonging to the global clones, the distribution of OC locus forms in these groups was examined first. Four of the additional complete genomes [GenBank accession numbers CP003856, CP003846, CP003849, and AP01357] were from GC2 isolates and these all carried OCL1. Initially, the GC1 and GC2 draft genomes were searched for the presence of the characterized OCL1, OCL2 and OCL3 gene clusters. In all of the GC1 genomes and in 88 of the GC2 genomes, the OC locus was in a single contig or in 2 contigs that could be directly abutted. Most of the GC1 genomes carried OCL1 (9) or OCL3 (14), which had been seen previously. However, OCL2 was also found in 7 isolates, and the new OCL5 form was found in one ST19 isolate, OIFC074 [GenBank accession AMDE01000046] (Table 1). OCL5 is described in detail below. The GC2 chromosomes carried OCL1 (73) or OCL3 (15), indicating that the gene cluster in the OC locus has also been replaced at least once in this clone.

In the remaining 12 GC2 genomes, the OC locus was interrupted by an insertion sequence (IS), and the final distribution of OCL1 and OCL3 in GC2 is shown in Table 1. The IS was identified by matching the sequence at the ends of the appropriate contigs to sequences available in ISFinder. In 2 isolates carrying OCL3 [GenBank accessions AMSX01000028 and AMHK01000047], ISaba13 interrupted gtrOC10 at the same position. The locations of IS in the 10 isolates with OCL1 is shown in Figure 2B with the identity of the IS and the number of isolates with a specific insertion indicated below. In those cases where a gene is interrupted, it would be expected that the OC structure will be altered as a consequence, as has been reported recently for other IS insertion mutants [16].

**Distribution of OCL1, OCL2 and OCL3 in other Acinetobacter baumannii genomes**

The remaining complete genomes [GenBank accession numbers NC_021733 and AERZ01000079] both included OCL2, and in one of them, NC_021733, an ISaba20 element interrupted the weeB gene. Eight draft genomes were from ST3 isolates (Table S3) and all included OCL1. The remaining 96 draft genomes were screened for the presence of OCL1, OCL2 and OCL3. 35 isolates belonging to 15 different STs included OCL1, indicating that OCL1 is very widely distributed (Table 1). Two isolates from ST10 carried OCL2, and a single ST136 isolate carried OCL3.
Further OC gene clusters

The remaining 53 genomes were examined individually and nine additional OC locus types were found. The open reading frames (ORFs) detected in the new configurations were characterized using Pfam and clan assignments, and these are shown in Table 2. Genes identified were named according to the annotation scheme developed previously (Table 3). Each new locus form was numbered (OCL4-OCL12) in the order identified. The most common were not confined to a specific ST (Table 1). These were OCL5 (12 isolates, 7 STs), OCL6 (20 isolates, 16 STs), OCL7 (6 isolates, 5 STs), and OCL8 (6 isolates, 3 STs). The remaining types were found in only 1 to 3 isolates (Table 1 and Table 4).

OCL1, OCL2 and OCL3 share 6 genes adjacent to *ilvE*, and 4 of the new locus forms were clearly related sharing a minimum of 5 of those 6 genes (Figure 3A). This group was designated Group A. Most of the remaining OC gene clusters formed a separate group, Group B (Figure 4), and OCL5, which appears to be a hybrid with a further type, was most closely related to this group. Although Group B gene clusters are also principally composed of *gtrOC* genes, only the first gene, *gtrOC1*, is shared by both groups suggesting that it may catalyze the first linkage between the inner and outer core. Pairwise nucleotide and protein identities for *gtrOC1* and GtrOC1 of Group A and B are >90%. The differences in genetic composition within and between Groups A and B are described in detail below.

**Group A**

Each Group A locus configuration contains between 6 to 8 putative *gtrOC* genes, as well as a *pda1* gene that predicts a polysaccharide decacetylation that enables production of D-galactosamine (D-Gal\(_p\)N) and D-glucosamine (D-Glc\(_p\)N), and an *orf1* gene (gly in [12], see Table 2) previously predicted to encode a glycosyl hydrolase (Figure 3). Four further variants were found but were rare, with OCL10 in 3 isolates (2 ST79) and OCL4, OCL11 and OCL12 in one isolate only. OCL10 and OCL11 share the first six genes with OCL1, OCL2 and OCL3, and all pairwise DNA identities are >98% over this region, but each carry different genes between *orf1* and *aspS*. OCL10 contains an additional nucleotide-linked sugar biosynthesis gene. The *glf* gene predicts a protein with 61% identity to Glf UDP-D-Galactopyranose [UDP-D-GalβN] and D-glucosamine [D-GlcβN], and an *orf1* gene (*gly* in [12], see Table 2) previously predicted to encode a glycosyl hydrolase (Figure 3). Two variants, OCL4 and OCL12, differ from OCL1 and all other Group A members in a 1.25 kb segment that includes *gtrOC12* and ~260 bp of an *orf1* gene, and replaces *gtrOC4* and the 3’ end of *orf1* (Figure 3A). OCLA exhibits high identity to

---

**Figure 2. A. baumannii** OCL1, OCL2 and OCL3 gene clusters. A. OCL1, OCL2 and OCL3 arrangements are from [12]. Arrows indicate genes and the direction of transcription. Gene names are shown above, colour scheme key is below, and shared portions are boxed. The gene named *orf1* was *ghy* in [12] (see Table 2). B. Location of IS elements in sequenced OCL1 variants. Positions of IS elements are shown below. GenBank accession numbers of OCL1 variants: APBE01000071 and APBF01000005 (OCL1m), APBJ01000096 (OCL1n), ANNC01000028 (OCL1o), AMDQ01000121 (OCL1p), AMIF00000000, AMIG00000000 and AMIU00000000 (OCL1q), AFDO01000006 and ACYS02000025 (OCL1r). doi:10.1371/journal.pone.0107833.g002
Table 1. The distribution of OC locus forms found in multiple STs\textsuperscript{a}\textsuperscript{b} in the draft genomes.

| OCL1 | OCL2 | OCL3 | OCL5 | OCL6 | OCL7 | OCL8 | OCL9 | OCL10 |
|------|------|------|------|------|------|------|------|-------|
| ST1  (8) | ST1  (5) | ST1 (14) | ST19 (1)\textsuperscript{c} | ST6 (1) | ST15 (1) | ST49 (2) | ST218 (1) | ST79 (2) |
| ST2 (82) | ST10 (2) | ST2 (17) | ST25 (5) | ST9 (1) | ST16 (1) | ST164 (1) | NEW-1 (1) | NEW-7 (1) |
| ST3  (8) | ST81 (2)\textsuperscript{c} | ST136 (1) | ST37 (1) | ST12 (1) | ST113 (2) | ST241 (3) | NEW-4 (1) |
| ST11 (1) | ST136 (1) | ST25 (2) | ST221 (1) |
| ST19 (1)\textsuperscript{f} | ST155 (1) | ST32 (3) | NEW-14 (1) |
| ST20 (2) | ST215 (2) | ST33 (1) |
| ST47 (1)\textsuperscript{g} | ST372 (1) | ST34 (1) |
| ST52 (2) | ST36 (1) |
| ST78 (4) | ST38 (1) |
| ST158 (1) | ST40 (1) |
| ST255 (1) | NEW-3 (2) |
| NEW-2 (1)\textsuperscript{f} | NEW-9 (1) |
| NEW-5 (2) | NEW-11 (1) |
| NEW-6 (1) | NEW-15 (1) |
| NEW-8 (7) | NEW-16 (1) |
| NEW-10 (2) | NEW-17 (1) |
| NEW-12 (8) |
| NEW-13 (1) |

\textsuperscript{a} ST is sequence type.  
\textsuperscript{b} Number of isolates of each ST shown in brackets.  
\textsuperscript{c} SLV of ST1.  
\textsuperscript{d} SLV of ST2.  
\textsuperscript{e} NEW is an ST with known alleles but an unrecorded MLST profile.  
\textsuperscript{f} doi:10.1371/journal.pone.0107833.t001
| Protein | GenPept accession | Annotations of BLASTp matches | Pfam | Clan |
|---------|-------------------|------------------------------|------|------|
| **Glycosyltransferases** | | | | |
| GtrOC1\* | AGK44465 | Hypothetical/Glycosyltransferase | Mito_fiss_Elm1 | CL0113 |
| GtrOC2 | AGK44464 | Glycosyltransferase | Glyco_trans_1,2 | CL0113 |
| GtrOC3 | AGK44462 | Glycosyltransferase | Glycos_transf_2 | CL0110 |
| GtrOC4 | AGK44461 | Glycosyltransferase | Glycos_transf_1, Glyco_transf_4 | CL0113, CL0113 |
| GtrOC5 | AGK44459 | Glycosyltransferase | Glyco_transf_25 | CL0110 |
| GtrOC6 \^b | AGK44458 | Hypothetical | n/a | n/a |
| GtrOC7 \^b | AGK44457 | Glycosyltransferase | n/a | n/a |
| GtrOC8 | AHK10024 | Glycosyltransferase | Glyco_transf_25 | CL0110 |
| GtrOC9 | ABO13302 | Glycosyltransferase | Gly_transf_sug, Gb3_synth | CL0110, n/a |
| GtrOC10 | AHK10230 | Glycosyltransferase | Glyco_transf_8, Glyco_transf_8C | CL0110, n/a |
| GtrOC11 | AHK10229 | Glycosyltransferase | Glyco_transf_2, Glyco_transf_4, Glyco_transf_1 | CL0113, CL0113 |
| GtrOC12 | EPG40646 | Glycosyltransferase | Glyco_transf_4, Glyco_transf_1 | CL0113, CL0113 |
| GtrOC13 | AHK10235 | Glycosyltransferase | Glyco_transf_2, Glyco_transf_4, Glyco_transf_1 | CL0113, CL0113 |
| GtrOC14 | AHK10234 | Hypothetical | Mito_fiss_Elm1 | CL0113 |
| GtrOC15 | AHK10233 | Glycosyltransferase | Glyco_transf_8, Glyco_transf_1 | CL0113, CL0113 |
| GtrOC16 | AHK10230 | Glycosyltransferase | Glyco_transf_8, Glyco_transf_8C | CL0110, n/a |
| GtrOC17 | AHK10229 | Glycosyltransferase | Glyco_transf_1,2 | CL0113 |
| GtrOC18 | EKU52820 | Glycosyltransferase | Glyco_transf_4, Glyco_transf_1 | CL0113, CL0113 |
| GtrOC19a | EKU52938 | Hypothetical/Glycosyltransferase | Mito_fiss_Elm1 | CL0113 |
| GtrOC19b | EKP44745 | Hypothetical/Glycosyltransferase | Mito_fiss_Elm1 | CL0113 |
| GtrOC19c | EKK07863 | Hypothetical/Glycosyltransferase | Mito_fiss_Elm1 | CL0113 |
| GtrOC20 | EKU52965 | Glycosyltransferase | Glyco_transf_2 | CL0110 |
| GtrOC21 | EKU52883 | Hypothetical/Glycosyltransferase | Glyco_transf_1,2, DUF3880 | CL0113, n/a |
| GtrOC22 | EKP65514 | Hypothetical/Glycosyltransferase | Mito_fiss_Elm1 | CL0113 |
| GtrOC23 | EKP65507 | Glycosyltransferase | Glyco_transf_4, Glyco_transf_1 | CL0113, CL0113, CL0113 |
| GtrOC24 | EKP65575 | Glycosyltransferase | Glyco_transf_2 | CL0110 |
| GtrOC25 | EKP65473 | Glycosyltransferase | Glyco_transf_1 | CL0113 |
| GtrOC26 | EKP44834 | Glycosyltransferase | Glyco_transf_2 | CL0110 |
| GtrOC27 | EKK07911 | mannosyltransferase | Caps_synth | CL0110 |
| GtrOC28 | EKK07913 | Glycosyltransferase | Glyco_transf_2,5 | CL0110 |
| GtrOC29 | WP_000760330 | Glycosyltransferase | Glyco_transf_25 | CL0110 |
| GtrOC30 | WP_000760349 | Glycosyltransferase | Glyco_transf_25 | CL0110 |
| GtrOC31 | WP_024432898 | Glycosyltransferase | Glyco_transf_25 | CL0110 |
| **Other** | | | | |
| Ahy | EKP65391 | GDSL-like lipase/acylhydrolase family protein | Lipase_GDSL_2 | CL0264 |
| AtrOC1 | AHK10232 | Acyltransferase | Acyl_transf_3 | CL0316 |
| Glf | EPG40651 | Glf, UDP-galactopyranose mutase | GLF, NAD_binding_8 | CL0063, CL0063 |
| (HtrL) | AHK10231 | HtrL/YibB protein | HtrL_YibB | n/a |
| Orf1 \^c | AGK44460 | Hypothetical protein | n/a | n/a |
| Orf2 | EKP65421 | Hypothetical | n/a | n/a |
| Orf3 | WP_000793090 | Hypothetical | DUF707 | n/a |
| Pda1 | AGK44463 | Polysaccharide deacetylase | Polysacc_deac_1 | CL0158 |
| Pda2 | EKP44902 | Polysaccharide deacetylase | Polysacc_deac_1 | CL0158 |
| Pda2a | AHK10236 | Polysaccharide deacetylase | Polysacc_deac_1 | CL0158 |
| PtoOC1 | WP_024432898 | Glycosyltransferase/pyruvyltransferase/exosortase | PS_pyruv_trans | CL0113 |
| RmlA | EKU52850 | RmlA, glucose-1-phosphate thymidylyltransferase | NTP_transferase | CL0110 |
| RmlB | EKU52300 | RmlB, dTDP-glucose 4,6-dehydratase | Epimerase | CL0063 |
| RmlC | EKU52972 | RmlC, dTDP-4-dehydrorhamnose 3,5-epimerase | dTDP_sugar_isom | CL0029 |
| RmlD | EKU52914 | RmlD, dTDP-4-dehydrorhamnose reductase | RmlD_sub_bind | CL0063 |
OCL1 over the remaining 7.39 kb of the 8.65 kb locus. Likewise, OCL10 and OCL12 share high identity over the majority of the gene cluster, differing only in the 1.25 kb region (Figure 3B). GtrOC4 and GtrOC12 are only 38% identical and, assuming that both enzymes are functional, would be expected to form different linkages possibly between the same sugar substrates. However, the predicted 294 aa Orf1 proteins [GenPept AGK44820 and EPG40642 – EPG40653] share 88% identity overall but are >95% identical over the first 208 aa, and 69.7% in the remaining 86 aa.

The OCL12 gene cluster was detected only in the draft genome of a single ST40 isolate, A. baumannii strain NIPH 410 [WGS accession ATG01000006; GenPept EPG40642 – EPG40653]. The arrangement appears to be a hybrid containing two arms that are much like parts of OCL4 and OCL10 (Figure 3B), sharing >98% identity with their counterparts. OCL4 and OCL12 share the region including gtrOC1 to orf1, and the 5′-end of gtrOC5 and gtrOC29. The first 100 aa of the predicted protein sequences of gtrOC5 and gtrOC29 share 95% identity but the other 150 aa are only 44.7% identical. Recombination between OCL4 and OCL10 in approximately 1 kb spanning part of orf1 and the shared 5′-end of gtrOC5/gtrOC29 would give rise to the OCL12 gene cluster.

**Group B**

The OC forms belonging to Group B (Figure 4, Table 5) share a smaller proportion of common sequence adjacent to ilvE (2, 3 or 4 genes) than the configurations in Group A. Each Group B form contains 5 to 6 gtrOC genes, as well as the pda2 gene that is predicted to encode a polysaccharide deacetylase (Table 2). Despite being characterized as putative polysaccharide deacetylases based on BLASTp hits and protein family (Pfam) predictions (Table 2), Pda1 (Group A) and Pda2 (Group B) do not share significant sequence identity. In four of the five Group B gene clusters, the pda2 genes are 99% identical. However, in OCL5, the pda2a gene is a hybrid, with the first half (323 bp) 96.7% identical to pda2 in the other four and the second half (364 bp) significantly diverged (68.1% identity). Hence, OCL5 may be a hybrid with a further form.

In OCL6, OCL8 and OCL9, the shared portion extends to near the 3′-end of gtrOC19 with 3 different sequences constituting the remainder of the gene. The three predicted GtrOC19 proteins designated a, b or c share 84–86% identity overall but are 95% identical over the first 283 aa of the total 345 aa indicating that recombination has occurred within this gene on at least two occasions. Despite this, the three GtrOC19 proteins may catalyze the same linkage in the oligosaccharide structures. OCL6, OCL8 and OCL9 also share the last gene in the cluster, gtrOC21, and the predicted GtrOC21 proteins are >96% identical. The gtrOC20 gene in OCL6 and gtrOC26 in OCL8 are also related but share only 84% DNA identity.

The OCL5 and OCL7 gene clusters include potential sugar modification genes. OCL5 contains an atrOC gene predicted to encode an acyltransferase (Table 2). OCL5 also includes a gene that predicts a protein that belongs to the HtrL_YibB protein family, which includes uncharacterized proteins from Salmonella enterica, Escherichia coli and Campylobacter jejuni that are also associated with LOS biosynthesis. OCL7 contains an additional ahv gene for a predicted acyl hydrolase.

In OCL6, the second most abundant form found in the genomes analyzed (Table 1), is unique in that it includes a dTDP-L-Rhamnose (dTDP-L-Rhap) biosynthesis operon. OCL6 is the only one to contain genes required for the synthesis of a complex sugar precursor. The rmlB, rmlD, rmlA and rmlC genes are arranged in a module that is located between gtrOC19a and gtrOC20 in OCL6. The four predicted Rml proteins share more than 53% identity with Rml proteins encoded in a Pseudomonas aeruginosa PA01 gene cluster [GenBank accession NC_002516]. In P.

| Table 2. Cont. |
|----------------|
| Protein | GenPept accession | Annotations of BLASTp matches | Pfam | Clan |
|---|---|---|---|---|
| WecB | ABO13303 | UDP-N-acetylgalactosamine 2-epimerase | Epimerase_2 | CL0113 |

Table 3. Key to gene names used.

| Gene name | Predicted protein | Predicted reaction product |
|---|---|---|
| ahv | Acyl- or Acetyl- hydrolase | - |
| atrOC | Acyl- or Acetyl- transferase (outer core) | - |
| gfl | UDP-D-galactopyranose mutase | UDP-D-GalF |
| gtrOC | Glycosyltransferase (outer core) | - |
| pda | Polysaccharide deacetylase | UDP-D-GlcPN |
| ptr | Pyruvyltransferase | - |
| rml | Multiple | dTDP-L-Rhamnosep |
| wecB | UDP-D-GlcPNAc C2 epimerase | UDP-D-ManpNAC |

doi:10.1371/journal.pone.0107833.t003

Acinetobacter baumannii OC Locus Variations

PLOS ONE | www.plosone.org 6 September 2014 | Volume 9 | Issue 9 | e107833
Table 4. OC locus forms found in single draft genomes.

| OCL  | ST** | GenBank Accession* |
|------|------|-------------------|
| OCL4 | NEW-18 | ASER01000014 |
| OCL11 | NEW-19 | ASFV01000000 |
| OCL12 | ST40 | ATGJ01000006 |

* ST is sequence type – Institut Pasteur scheme.
** NEW is ST with known alleles but an unregistered MLST profile.
*** The accession number is for the contig containing the OC locus.

Figure 3. Group A OC locus arrangements. A. Genes are shown as arrows indicating the direction of transcription. The colour scheme represents the predicted functional family of the gene products and is shown below. Assigned gene names are shown above as defined in Table 3. Shared regions are boxed. B. Comparison of OCL12, OCL4 and OCL10. Grey shading indicates shared regions, with the % nucleotide identity between pairs shown. Figures are drawn to scale from GenBank accession numbers JN968483 (OCL1), CP000521 (OCL2), CP001182 (OCL3), ASER01000014 (OCL4), AMHC01000037 (OCL10), ASFV01000000 (OCL11), and ATGJ01000006 (OCL12).

doi:10.1371/journal.pone.0107833.t004
doi:10.1371/journal.pone.0107833.g003
These genes have been shown to direct the synthesis of dTDP-L-Rhap (Figure 5) [27]. The rml genes are found in the rmlBDAC organization in many bacterial species including *E. coli* and *S. enterica* [28]. The ability to synthesize this sugar suggests that OCL6 may generate the L-Rhap-containing OC structure in Figure 1.

**Detection of OC locus variants**

Differences at the OC locus have the potential to assist in understanding the epidemiology of *A. baumannii* by detecting variation in otherwise related isolates. Hence, a step-wise PCR approach was developed to facilitate the detection of OC locus types. Short-range PCRs specific for *pda1* or *pda2* were first used to distinguish Group A and Group B gene clusters. To confirm this assignment, the reverse primers in *pda1* or *pda2* were used in conjunction with primer RH1701 to amplify the *ilvE* proximal portion that is conserved in each group (Figure 6, Table 6). Following this, generic long-range PCRs linking either *pda1*-aspS or *pda2*-aspS were used to amplify the right arm of the assigned group. As these PCRs yield large products, restriction digestion was used to differentiate specific gene clusters. The PCRs were validated using genomic DNA from sequenced isolates from our own collection that are known to carry OCL1, OCL2, OCL3, and OCL4 for Group A, and OCL5, OCL6, and OCL8 for Group B (data not shown).

To facilitate the rapid detection of the OC locus types that were found in the majority of isolates belonging to the multiply antibiotic resistant GC1 and GC2 clones, short-range PCRs specific for OCL1, OCL2, OCL3, or OCL5 were also developed (Table 6). The specificity of these PCRs was validated using the OCL representatives listed above (data not shown).

**SMAL carries OCL1**

Since SMAL produces the OC1 configuration of LOS [21], the PCR scheme was used to determine if, as predicted, this isolate carries the OCL1 gene cluster. The OCL1-specific PCR (Table 6) detected a product of the expected size, confirming the presence of OCL1 in SMAL. In addition, both the left and right arms were amplified using overlapping PCRs linking *ilvE* to gtrOC5 (primer RH1701 with RH1704) and gtrOC4 to aspS (primer RH1705 with RH1702) and both reactions yielded products of the expected size. However, given that gtrOC5 is also present in OCL4, the left-arm PCR was digested with *Xba*I, which yielded fragments of 757, 879, 2009, 2730 bp as predicted for OCL1, whereas OCL4 would produce fragments of 757, 879, 4714 bp.

**Table 5. GenPept accessions for representative Group B OC locus forms.**

| OCL  | WGS accession | OCL peptides        |
|------|---------------|---------------------|
| OCL5 | AFDL010000002 | EJG21764 – EJG21756 |
| OCL6 | AMFW010000007 | ELX01369 – ELX01508 |
| OCL7 | AMTB01000038  | EKP65593 – EKP65458 |
| OCL8 | AMFY01000013  | EKP44739 – EKP44897 |
| OCL9 | AMFI01000027  | EKK07813 – EKK07789 |

doi:10.1371/journal.pone.0107833.t005
Identification of WaaL homologues in non-
baumannii species

In some Gram-negative bacteria, the complex carbohydrate polymer that is exported as capsule can be also ligated to the LOS as the O antigen to form LPS. This reaction is catalyzed by a WaaL O-antigen ligase. Previously, the absence of a waaL gene in 10 complete A. baumannii genomes had been determined using BLASTp searches with Pseudomonas aeruginosa and E. coli WaaL queries [12]. However, two WaaL homologues [GenPept accessions WP_005244245 and WP_010112638] were identified in non-baumannii species but their location was not reported. As expected, the waaL genes are in the vicinity of the OC locus, located between aspS and a gene encoding a TonB-dependent receptor, and separated from aspS by a single open reading frame. Here, the BLASTp search was repeated using each of these WaaL sequences as queries. There were no significant hits in any A. baumannii derived GenBank entry for which translations were available. However, homologues of WP_005244245 were found in several more recently released genomes of other Acinetobacter species (Table 7), and all of them were between aspS and tonB. The sequence between aspS and the TonB-dependent receptor gene was also checked in all A. baumannii draft genomes, and no additional genes were found in this location. These findings confirm our previous conclusion that A. baumannii strains do not produce LPS.

Discussion

Variation at the OC locus has been reported in several Gram-negative species. E. coli has five different forms [29], and more extensive variation has been reported for Campylobacter jejuni (19 types; [30]) and Neisseria meningitidis (11 types; [31]). In this study, nine novel OC gene cluster types were discovered in complete and draft A. baumannii genome sequences adding to the three described previously [12], and bringing the total to twelve. This would lead to significant variation in the structure of the LOS on the cell surface, which could affect the various virulence properties associated with LOS in A. baumannii [2–7]. Additionally, the IS elements found in some OC gene clusters disrupt glycosyltransferase or modification genes and these would also alter the final OC structure.

A notable finding of this study was the identification of a second group of related OC arrangements, Group B. Group B locus forms are unified by shared genes adjacent to ilvE, as are Group A forms. Within Groups A and B, there are examples of both small and large regions of sequence difference between pairs of OC configurations. These replacements generally do not include a complete gene or genes. In cases where one gene was replaced with another (gtrOC4 and gtrOC12), the recombination patch

![Figure 5. Genes for dTDP-L-Rhamnose synthesis.](https://example.com/figure5)

![Figure 6. PCR scheme for typing Group A and Group B OC gene clusters.](https://example.com/figure6)
extended into adjacent genes, and whether the products of these genes retain their activity or specificity will need to be determined either experimentally or by determining the OC structure. Evidence is mounting to support the conclusion that Acinetobacter baumannii does not produce LPS [12–17], and here an additional analysis failed to find a gene for a homologue of WaaL. However, a number of genomes of other Acinetobacter species were found to include a predicted waaL gene close to their OC locus. Of twelve different OC gene clusters, OCL1 and OCL6 were the most widespread, with OCL1 in 18 different STs and OCL6 in 16, indicating that recombinational exchange between A. baumannii strains is extensive. OCL1 corresponds to one of the solved LOS structures [12]. It is likely that OCL6 directs the synthesis of the second solved structure as it includes L-Rha residues (Figure 1) and only OCL6 contained genes for L-Rhap synthesis. Though genes located elsewhere, such as in the locus for capsule synthesis, could contribute this sugar precursor, rml genes are found only in a few capsule forms [32]. A link between OCL6 and the solved structure needs confirmation, either by sequencing the OC locus of strain ATCC 17904 [25] or by elucidating the LOS structure of a strain that carries OCL6.

Of twelve different OC gene clusters, OCL1 and OCL6 were the most widespread, with OCL1 in 18 different STs and OCL6 in 16, indicating that recombinational exchange between A. baumannii strains is extensive. OCL1 corresponds to one of the solved LOS structures [12]. It is likely that OCL6 directs the synthesis of the second solved structure as it includes L-Rhap residues (Figure 1) and only OCL6 contained genes for L-Rhap synthesis. Though genes located elsewhere, such as in the locus for capsule synthesis, could contribute this sugar precursor, rml genes are found only in a few capsule forms [32]. A link between OCL6 and the solved structure needs confirmation, either by sequencing the OC locus of strain ATCC 17904 [25] or by elucidating the LOS structure of a strain that carries OCL6.

The GC1 and GC2 multiply antibiotic resistant isolates for which genomes were available were from a range of sources, hospitals and countries, but most of them carried OCL1. However, we uncovered further variation at the OC locus in GC1 and for the first time, detected a different OCL in 17 of the 100 in GC2 isolates. OC diversity within the major global clones of A. baumannii indicates that this region is being exchanged or replaced repeatedly and more OCL may be found in these clones in the future. To facilitate tracking of these sub-lineages, a comprehensive OCL PCR-typing scheme was developed. Here, it was used to confirm that SMAL carries OCL1. However, this scheme will provide a simple means to distinguish otherwise closely-related strains involved in outbreaks and increase our understanding of the evolution and epidemiology of A. baumannii, particularly of the major global clones.

### Table 6. PCR scheme for the detection of OC locus arrangements.

| Target groups or forms | PCR type | Target gene(s) | Primers | Sequence (5’ – 3’) | Amplicon size |
|------------------------|----------|----------------|---------|--------------------|--------------|
| All                    | Generic  | ilvE – aspS    | RH1701  | GGCAGCTTGACGGTTTACA GGCAGCTTCAATTCA  | Various      |
| Group A                | Generic  | pda1           | RH1817  | GTGCCAGGTTTGGTTTTACGCAAGGCGCTGGCCATCG | 0.18 kb      |
| Group A                | Generic  | ilvE – pda1    | RH1817  | GGCAGCTTGACGGTTTACA GGCAGCTTCAATTCA  | 3.00 kb      |
| Group A                | Subspecific | pda2 - aspS     | RH1817  | GTGCCAGGTTTGGTTTTACGCAAGGCGCTGGCCATCG | Various      |
| OCL1, OCL4             | Subspecific | ilvE – gtrOC5  | RH1701  | GGCAGCTTGACGGTTTACA GGCAGCTTCAATTCA  | 6.38 kb      |
| OCL1, OCL2, OCL3, OCL10, OCL11 | Subspecific | gtrOC4 - aspS | RH1705  | CTCAGCGCCGTACITACAAC GGCAGCTTCAATTCA  | Various      |
| Group B                | Generic  | pda2           | RH1819  | GGGTCAAGGCCGCTGGATTTGTGCAACACGACATCG | 0.24 kb      |
| Group B                | Generic  | ilvE – pda2    | RH1817  | GGCAGCTTGACGGTTTACA GGCAGCTTCAATTCA  | 1.64 kb      |
| Group B                | Subspecific | pda2 - aspS     | RH1819  | GGGTCAAGGCCGCTGGATTTGTGCAACACGACATCG | Various      |
| OCL1                   | Specific  | gtrOC4 – gtrOC5 | RH1705  | CTCAGCGCCGTACITACAAC GGCAGCTTCAATTCA  | 1.93 kb      |
| OCL2                   | Specific  | wecB – gtrOC9  | RH1811  | GGGGTCAGATCAGGCTGGCCGCTGGCCATCG | 0.68 kb      |
| OCL3                   | Specific  | gtrOC10 – gtrOC11 | RH1813  | GCGCGGCGCTGGCCATCG | 0.99 kb      |
| OCL5                   | Specific  | gtrOC16 – gtrOC17 | RH1815  | GCAGCGACACTCCAGGGCT | 1.08 kb      |

### Table 7. WaaL homologues in the vicinity of the OC locus.

| Species                 | GenPept accession | % aa identity to WP_005244245.1 | % aa identity to WP_010112638.1 |
|-------------------------|--------------------|----------------------------------|----------------------------------|
| Acinetobacter sp.       | WP_005244245.1*   | 100                              | 31                               |
| Acinetobacter sp.       | WP_010112638.1*   | 31                               | 100                              |
| Acinetobacter sp.       | WP_005269248.1    | 88                               | 29                               |
| Acinetobacter sp.       | WP_016162617.1    | 79                               | 31                               |
| Acinetobacter sp.       | WP_004802756.1    | 79                               | 31                               |
| Acinetobacter sp.       | WP_004652452.1    | 76                               | 31                               |
| Acinetobacter bereziniae| WP_004827805.1    | 48                               | 32                               |
| Acinetobacter rudis     | WP_016655105.1    | 47                               | 31                               |
| Acinetobacter gilouiae  | WP_004723110.1    | 49                               | 32                               |
| Acinetobacter gilouiae  | WP_004819073.1    | 48                               | 33                               |

* Reported in [12].
\(^{b}\) Identity for proteins with >91% coverage.

\(\text{doi:10.1371/journal.pone.0107833.t006}\)
Materials and Methods

Bioinformatic analysis

Strain information and accession numbers for the GC1, GC2 and ST3 isolates among the 234 A. baumannii genome sequences available on the 1st November 2013 and used in this analysis are listed in Table S1, Table S2 and Table S3. Information on the remaining isolates is in Table 1, Table 4 and Table 5. MLST profiles were extracted from the genomes using a script available at http://sourceforge.net/projects/srst/files/mlstBLAST/ , and assigned STs using the Institut Pasteur MLST scheme at http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html.

Genomes containing OCL matches of >97% identity to the full length of OCL1, OCL2 or OCL3, were identified with BLASTn using sequence queries from GenBank accession numbers JN968483 (OCL1), CP000521 (OCL2), and KC118540 (OCL3). Genomes matching parts of all three were assessed for other genes that were identified and characterized as described below. For the remaining genomes, the sequence located between the ilvE and aspS genes in each genome was located and analyzed as described previously [12]. As the majority of draft genomes available in WGS are unannotated and hence do not include predicted polypeptides, a representative of each novel locus form was assessed for ORFs using the ORF Finder tool (http://www.ncbi.nlm.nih.gov/projects/gorf/). Elements were identified using the IS Finder database (https://www-is.biotoul.fr//is.html). Proteins predicted from the sequences were analyzed using BLASTp [33] against the non-redundant GenBank database using default parameters, and only cases where polypeptides were co-linear and >25% identical were used for annotation. Predicted protein sequences were also submitted to the protein family (Pfam) database (http://pfam.xfam.org/) [34] and those that included the complete sequence or a domain using default parameters were assigned Pfam classifications. Annotation used an extension of the scheme developed in a previous study [12]. All gene identifiers used in this annotation scheme are summarized in Table 3. Predicted gtrOC, atrOC and pda sequences were assigned different numbers when the amino acid identity of the gene product was less than 85% to its closest homologue. When IS were present, the OCL locus type was considered a variant of a specific OCL and a letter was added after the OCL number.

PCR

Whole cell DNA was extracted from A. baumannii strains as described previously [35]. A. baumannii strain SMAL was kindly provided by Cristina De Castro (University of Napoli, Italy).

Primers (Integrated DNA Technologies Inc., San Diego), their targets, and expected amplicon sizes are listed in Table 6. Both short- and long-range PCR amplifications were carried out using genomic DNA as a template. The reaction mix (12.5 µL) for short-range PCR contained 1.25 µL of Thermopol PCR buffer, 12.5 pmol of each primer, 200 µM of each deoxynucleoside triphosphate, 40 ng of template DNA and 1 unit of Taq DNA polymerase (New England BioLabs, Ipswich, MA, USA). Thermocycling conditions were as follows: 94°C for 3 min (initial denaturation cycle), followed by 30 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 60 s to 3 min, then a final cycle of 72°C for 5 min. For long-range PCR, the reaction mix (20 µL) included 4 µL of HF buffer (Finnzymes, Thermo Fisher Scientific, Australia), 1 µmol of each primer, 200 µM of each deoxynucleoside triphosphate, 40 ng of template DNA and 1 unit of Phusion polymerase (Finnzymes, Thermo Fisher Scientific, Australia). Cycling conditions were 98°C for 30 s (initial denaturation cycle), followed by 35 cycles of 98°C for 10 s, 60°C for 30 s and 72°C for 30 s per 1 kb of expected product size, then a final cycle of 72°C for 10 min.

PCR products were separated by electrophoresis on 1% (w/v) agarose gels and stained with 5 mg/L ethidium bromide. PCR products were visualized with ultraviolet (UV) light and imaged using a GelDoc XR image analysis station (Bio-Rad, Hercules, CA, USA). A 1 kb DNA ladder (New England BioLabs) was used for a molecular size marker. The PCRs were validated using ATCC 19606 (OCL1), ATCC 17978 (OCL2) and AB0057 (OCL3) and isolates from our own collection that carry OCL4 (A388) [23], OCL5 (D13) [16], OCL6 (D46) [36], and OCL7 (J9) [37]. For confirmation of PCR amplicons, products were digested using XbaI as per the manufacturers instructions (New England Biolabs).

Supporting Information

Table S1 OC forms detected in the draft genomes of A. baumannii ST1 isolates.

Table S2 OC forms detected in the draft genomes of A. baumannii ST2 isolates.

Table S3 OC forms detected in the draft genomes of A. baumannii ST3 isolates.

Acknowledgments

The authors wish to thank Cristina De Castro for supplying A. baumannii strain SMAL.

Author Contributions

Conceived and designed the experiments: RMH. Performed the experiments: JJK SJN. Analyzed the data: JJK RMH. Wrote the paper: JJK RMH.

References

1. Zhang G, Meredith T, Kahne D (2013) On the essentiality of lipopolysaccharide to Gram-negative bacteria. Curr Opin Microbiol 16: 779–785. doi: 10.1016/j.mib.2013.09.007

2. Moffatt J, Harper M, Harrison P, Hale J, Vinogradov E, et al. (2010) Colistin resistance in Acinetobacter baumannii is mediated by complete loss of the lipopolysaccharide production. Antimicrob Agents Ch 54: 4971–4977. doi: 10.1128/AAC.00834-10

3. McQueary C, Kirkup B, Si Y, Barlow M, Actis L, et al. (2012) Extracellular stress and lipopolysaccharide modulate Acinetobacter baumannii surface-associated motility. J Microbiol 50: 434–443. doi:10.1007/s12275-012-1355-1

4. Soon R, Nairn R, Harper M, Adler B, Boyce J, et al. (2011) Effect of colistin exposure and growth phase on the surface properties of live Acinetobacter baumannii cells examined by atomic force microscopy. Int J Antimicrob Ag 38: 493–501. doi:10.1016/j.ijantimicag.2011.07.014

5. Lin L, Tan B, Pantapalangkoor P, Ho T, Baquir B, et al. (2012) Inhibition of LpxC protects mice from resistant Acinetobacter baumannii by modulating inflammation and enhancing phagocytosis. MBio 3: e00312. doi: 10.1128/MBio.00312-12

6. Luke N, Sauberan S, Russo T, Beanan J, Olson R, et al. (2010) Identification and characterization of a glycosyltransferase involved in Acinetobacter baumannii lipopolysaccharide core biosynthesis. Infect Immun 78: 2017. doi: 10.1128/IAI.00016-10

7. Ereditato C, Monocayo-Nieto O, Morgan R, Young M, Poxton I (2007) Acinetobacter baumannii lipopolysaccharides are potent stimulators of human monocye activation via toll-like receptor 4 signalling. J Med Microbiol 56: 165–171.

8. Beceiro A, Llober E, Aranda J, Bengoechea J, Doumith M, et al. (2011) Phosphoethanolamine modification of lipid A in colistin-resistant variants of Physiologically mediated changes in Acinetobacter baumannii OC Locus Variations
Acinetobacter baumannii

mediated by the pmrAB two-component regulatory system. Antimicrob Agents Chemother 55: 3370–3379. doi: 10.1128/AAC.00079-11

9. Pelletier M, Casella L, Jones J, Adams M, Zurawski D, et al. (2013) Unique structural modifications are present in the lipopolysaccharide from colistin-resistant strains of Acinetobacter baumannii. Antimicrob Agents Chemother 57: 4831–4840. doi: 10.1128/AAC.00865-13

10. Indriati Hood M, Becker K, Roux C, Dunman P, Skaer E (2013) Genetic Determinants of Intrinsic Collistin Tolerance in Acinetobacter baumannii. Infect Immun 81: 542–551. doi: 10.1128/IAI.00704-12

11. Heinrichs D, Yethon J, Whitfield C (1998) Molecular basis for structural diversity in the core regions of the lipopolysaccharides of Escherichia coli and Salmonella enterica. Mol Microbiol 30: 221–232.

12. Kenyon J, Hall RM (2013) Variation in the complex carbohydrate biosynthesis loci of Acinetobacter baumannii genomes. PLOS ONE 8: e62160. doi: 10.1371/journal.pone.0062160

13. Lees-Miller RG, Iwashkiw JA, Scott NE, Seper A, Vinogradov E, et al. (2013) A common pathway for O-linked protein-glycosylation and synthesis of capsule in Acinetobacter baumannii. Mol Microbiol 89: 616–630. doi: 10.1111/mmi.12300

14. Fregelino E, Gargiolo V, Lanerotta R, Zarrilli M, Holtz O, et al. (2011) Identification and structural determination of the capsular polysaccharides from two Acinetobacter baumannii clinical isolates, MG1 and SMAL. Carbohydr Res 346: 973–977. doi: 10.1016/j.carres.2011.03.024

15. Russo T, Lake N, Beanan J, Omeni R, Sanberan S, et al. (2010) The K1 capsular polysaccharide of Acinetobacter baumannii strain 307–0294 is a major virulence factor. Infect Immun 78: 3993. doi: 10.1128/IAI.00366-10

16. Kenyon J, Holt K, Pickard D, Dougan G, Hall R (2014) Insertions in the OCL1 locus of Acinetobacter baumannii lead to shortened lipopolysaccharides. Res Microbiol 165: 472–475. doi: 10.1016/j.resmic.2014.05.034

17. Kenyon J, Marzaioli A, Hall R, De Castro C (2014) Structure of the K2 capsule associated with the K2L2 gene cluster of Acinetobacter baumannii. Glycobiology 24: 554–563. doi: 10.1093/glycob/cwt024.

18. Di Nocera P, Rocco F, Giannouli M, Triassi M, Zarrilli R (2011) Genome organization and structural characterization of the capsular polysaccharides from two Acinetobacter baumannii clinical isolates, MG1 and SMAL. Carbohydr Res 346: 973–977. doi: 10.1016/j.carres.2011.03.024

19. Senchenkova S, Shashkov A, Shneider M, Arbatsky N, Popova A, et al. (2014) Structure of the capsular polysaccharide of Acinetobacter baumannii ACICU containing di-N-acetylneuraminic acid. Carbohydr Res 391: 89–92. doi: 10.1016/j.carres.2014.04.002

20. Vinogradov E, Di Nocera P, Rocco F, Giannouli M, Triassi M, Zarrilli R (2011) Genome organization of epidemic Acinetobacter baumannii strains. BMC Microbiol 11: 224. doi: 10.1186/1471-2180-11-224

21. Fregelino E, Fugazza G, Galano E, Gargiolo V, Landini P, et al. (2010) Complete lipooligosaccharide structure of the clinical isolate Acinetobacter baumannii, strain SMAL. Eur J Org Chem 2010: 1345–1352. doi: 10.1002/ejc.200901396

22. Karah N, Sundhjord A, Towner K, Samuelson O (2012) Insights into the global molecular epidemiology of carbapenem non-susceptible clones of Acinetobacter baumannii. Drug Resist Updat 15: 237–247. doi: 10.1016/j.drup.2012.06.001

23. Pou, V, Hamidian M, Hall RM (2012) Antibiotic-resistant Acinetobacter baumannii variants belonging to global clone 1. J Antimicrob Chemother, 67: 1039–1040. doi: 10.1093/jac/dkr386

24. Zarrilli Z, Pournaras S, Giannoudi M, Tsakris A (2013) Global evolution of multidrug-resistant Acinetobacter baumannii clonal lineages. Int J Antimicrob Agents 41: 11–19. doi: 10.1016/j.ijantimicag.2012.09.008

25. Vinogradov E, Petersen B, Thomas-Oates J, Duus J, Brade H, et al. (1998) Characterization of a novel branched tetrasaccharide of 3-desoxy-D-manno-oct-2-ulopyranosonic acid. J Biol Chem 273: 20122–20131.

26. Koplin R, Brison J, Whitfield C (1997) UDP-galactofuranose precursor required for formation of the lipopolysaccharide O antigen of Klebsiella pneumoniae serotype O1 is synthesized by the product of the rfbD gene. J Biol Chem 272: 4121–4129.

27. Rahim R, Burrows L, Monteiro M, Perry M, Lam L (2000) Involvement of the rml locus in core oligosaccharide and O polysaccharide assembly in Pseudomonas aeruginosa. Microbiology 146: 2801–2814.

28. Li Q, Reeves P (2000) Genetic variation of fTDP-L-rhamnose pathway genes in Salmonella enterica. Microbiology 146: 2933–2943.

29. Amor K, Heinrichs D, Friedlich E, Ziebell K, Johnson R, et al. (2000) Distribution of core oligosaccharide types in lipopolysaccharides from Escherichia coli. Infect Immun 68: 1116–1124.

30. Parker C, Gilbert M, Yuki N, Endz H, Mandrell R (2000) Characterisation of lipooligosaccharide-biosynthetic loci of Campylobacter jejuni reveals new lipooligosaccharide classes: evidence of mosaic organizations. J Bacteriol 190: 5681–5689. doi: 10.1128/JB.00524-08

31. Scholten R, Kuipers B, Valkenburg H, Dunkerts J, Zollinger W, et al. (1994) Lipooligosaccharide immunotyping of Neisseria meningitidis by a whole-cell ELISA with monoclonal antibodies. J Med Microbiol 41: 236–243.

32. Hu D, Lui B, Dijkshoorn L, Wang L, Reeves PR (2013) Diversity in the major lipooligosaccharide types in lipopolysaccharides from Salmonella enterica. Mol Microbiol 89: 816–830. doi: 10.1111/mmi.12300

33. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403–410.

34. Nigro SJ, Post V, Hall RM (2011) Aminoglycoside resistance in multiply antibiotic-resistant Acinetobacter baumannii belonging to global clone 2 from Australian hospitals. J Antimicrob Chemother 66: 1504–1509. doi: 10.1093/jac/dkr163

35. Hamidian M, Hall RM (2013). ISAba1 targets a specific position upstream of the intrinsic ampC gene of Acinetobacter baumannii leading to cephalosporin resistance. Journal of Antimicrobial Chemotherapy, 68(11), 2681–2683. doi: 10.1093/jac/dkt233

36. Hamidian M, Hall RM (2014) Tsu6/68, a transposon carrying an ISAba1-activated ampc gene and conferring cephalosporin resistance in Acinetobacter baumannii. Journal of Antimicrobial Chemotherapy, 69, 77–80. doi: 10.1093/jac/dkt312