

K19 capsular polysaccharide of *Acinetobacter baumannii* is produced via a Wzy polymerase encoded in a small genomic island rather than the KL19 capsule gene cluster

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Polymerization of the oligosaccharides (K units) of complex capsular polysaccharides (CPSs) requires a Wzy polymerase, which is usually encoded in the gene cluster that directs K unit synthesis. Here, a gene cluster at the *Acinetobacter* K locus (KL) that lacks a *wzy* gene, KL19, was found in *Acinetobacter baumannii* ST111 isolates 28 and RBH2 recovered from hospitals in the Russian Federation and Australia, respectively. However, these isolates produced long-chain capsule, and a *wzy* gene was found in a 6.1 kb genomic island (GI) located adjacent to the *cpn60* gene. The GI also includes an acetyltransferase gene, *atr25*, which is interrupted by an insertion sequence (IS) in RBH2. The capsule structure from both strains was $\rightarrow 3\text{-}\alpha\text{-D-GalpNAc-(1}\rightarrow 4\text{)-}\alpha\text{-D-GalpNAcA-(1}\rightarrow 3\text{)-}\beta\text{-D-QuipNAc4NAc-(1}\rightarrow$, determined using NMR spectroscopy. Biosynthesis of the K unit was inferred to be initiated with QuiNAc4NAc, and hence the Wzy forms the $\beta\text{-(1}\rightarrow 3\text{)}$ linkage between QuiNAc4NAc and GalpNAc. The GalpNAc residue is 6-O-acetylated in isolate 28 only, showing that *atr25* is responsible for this acetylation. The same GI with or without an IS in *atr25* was found in draft genomes of other KL19 isolates, as well as ones carrying a closely related CPS gene cluster, KL39, which differs from KL19 only in a gene for an acyltransferase in the QuiNAc4NR synthesis pathway. Isolates carrying a KL1 variant with the *wzy* and *atr* genes

Received 24 March 2016

Accepted 24 May 2016

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Abbreviations: CPS, capsular polysaccharide; GalNAc, *N*-acetylgalactosamine; GalNAcA, *N*-acetylgalactosaminuronic acid; GlcNAc, *N*-acetylglucosamine; HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear single-quantum coherence; KL, K locus; QuiN4N, 2,4-diamino-2,4,6-trideoxy-D-glucose; ROESY, rotating-frame nuclear Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy; UDP, uridine diphosphate; UndP, undecaprenyl phosphate; ST, sequence type.

The GenBank/EMBL/DDJB accession number for the sequences of the KL19 gene clusters and the *wzy*-containing genomic island for RBH2 and 28 are KU165787 and KU215659, respectively.

One supplementary table is available with the online Supplementary Material.