

**A proposed novel high-resolution core genome multi-locus sequence typing (MLST) scheme for *Acinetobacter baumannii***

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**Objectives:** *Acinetobacter baumannii* is a therapeutic and infection control challenge worldwide. Currently, two MLST schemes are available for characterisation of *A. baumannii* population structure (<http://pubmlst.org/abaumannii/> & <http://www.pasteur.fr/mlst/Abaumannii.html>) but their applicability for investigation of nosocomial outbreaks is suboptimal. Microbial whole genome sequencing (WGS) currently allows bacterial typing based on a gene-by-gene approach utilising a large set of core genome genes as compared to only seven housekeeping genes in traditional MLST, thus achieving a refined population structure of important pathogens. Here we propose a novel core genome MLST (cgMLST) typing scheme for *A. baumannii*.

**Methods:** For the construction of the cgMLST scheme we downloaded from NCBI, 16 published *A.baumannii* reference genomes. Processing via RAST/CD-HIT software and an in-house bioinformatics pipeline identified a set of single copy core genes, common to all strains. To validate the scheme, 20 unpublished *A. baumannii* blood isolates recovered in a single hospital were subject to WGS using Illumina Miseq (PE 250bp). Draft genome data was generated using SPAdes Genome Assembler. MLST Sequence types (ST) were assigned from the assemblies per both pubMLST and Pasteur schemes. A gene allele calling script was developed and applied to define standardised alleles for all analysed genomes, thus creating a novel typing cgMLST scheme.

**Results:** The initial gene set derived from reference genomes (1,223 loci) was narrowed down to 1,048 loci present in all draft genomes, resulting in calling of new alleles for the majority of loci. All 20 draft genomes generated unique allelic profiles. The novel cgMLST approach discriminated STs of analysed strains per both MLST schemes. Notably, cgMLST revealed that Pasteur ST2 harbours strains with up to 31% difference in allelic pattern. A goeBURST Minimum Spanning tree (MST) coloured by the ST defined by pubMLST scheme, demonstrated existing diversity between strains with identical ST (Fig 1). ST457 (50% of strains, light green) and ST106 (20% of strains, orange) appeared in clusters that had only up to 7% and 1% difference in the allelic profiles, respectively, while three strains with ST218 (1 draft genome and 2 NCBI, dark green) exhibited up to 18% difference in allelic profile.

**Conclusion:** The proposed cgMLST scheme offered maximum discrimination between *A. baumannii* strains whilst overcoming some caveats of current MLST schemes. cgMLST approaches allow for standard and portable methodology, common allelic nomenclature and easy implementation in automated analysis pipelines, and are thus expected to enable the adoption of draft genome sequencing as a mainstream procedure in clinical microbiology. The proposed scheme will be further refined via analysis of additional draft genomes and through different draft genome determination pipelines and is envisioned to become a publicly available tool facilitating standardised comparison of *A. baumannii* draft genomic data.

**goeBURST MST of *Acinetobacter baumannii* cgMLST(1048 loci)**

Colours represent different STs by pubMLST scheme  
 Nodes without ID: NCBI strains  
 Nodes with ID: draft genome strains  
 Numbers in link labels represents number of allele differences

