High Resolution Clonality of Outbreak-Causing *Acinetobacter baumannii* studied by Whole Genome Mapping

Sabirova J.¹, Xavier B.B.¹, Coppens J.¹, Lammens C.¹, Dafopoulos K.², Zarkotou O.², Wagner T.⁵, Pournaras S.³, Tsakris A.³, Goossens H.¹, Malhotra-Kumar S.¹

Department of Medical Microbiology, Vaccine & Infectious Disease Institute, University of Antwerp, Antwerp, Belgium¹ - Department of Microbiology, Tzaneio General Hospital, Piraeus²

Department of Microbiology, Medical School, University of Athens, Athens, Greece³ - Medical School, University of Thessaly, Larissa⁴ - OpGen, Inc., Gaithersburg, USA⁵

**Introduction**

Whole Genome Mapping (WGM) is a valuable molecular tool for high resolution clonality of microbial pathogens¹. WGM has been successfully employed in epidemiological outbreak studies when strains needed to be rapidly typed, and in a high throughput manner, for comparisons to related outbreak/non-outbreak strains. Here we employed WGM to study clonality of 16 isolates of *A. baumannii* isolated from Greek patients during an outbreak (2008) and in a 'non-outbreak' situation (2008-2013) at Tzaneio Hospital, Athens and University Hospital of Larissa, Greece.

Figure 1A: Comparison of whole genome maps of typed *A. baumannii* in Bionumerics

**Methods**

Non-outbreak (n=13, NO1-NO13) and outbreak (n=3, O1-O3) *A. baumannii* strains were typed by multi-locus sequence typing (MLST) prior to mapping by WGM. For WGM, high molecular DNA was prepared using agarose plugs. NcoI restriction maps were generated on the Argus® system (Opgen, Gaithersburg, USA) and analysed using Bionumerics (Applied Maths, Belgium). Antimicrobial resistance profiles were determined by disk diffusion.

**Conclusions**

This study revealed high genomic heterogeneity of the typed *A. baumannii* clinical isolates (SR of ≥44%) with only marginal differences detected between the closest typed outbreak and non-outbreak strains. Transition of *A. baumannii* from a non-outbreak to an outbreak strain is thus likely to involve acquisition of plasmids, SNPs and/or other point chromosomal or plasmid-encoded mutations, with all of these changes not detectable by MLST.

**Results**

According to MLST, all typed *A. baumannii* belonged to CC2 (http://www.pasteur.fr/recherche/genopole/PF8/mlst/). According to WGM, the strains formed three distinct clusters: C1 (outbreak-causing O1, O2, and O3, non-outbreak NO1-NO5), C2 (non-outbreak NO7-NO13) and C3 (non-outbreak NO6) (Fig.1A). The inter-cluster similarity rate (SR) was 74% for isolates belonging to C1 and C2, whereas only 44% of inter-cluster SR was detected for C3 as compared to C1/C2 clusters (Fig. 1A). The intra-cluster SR was 93% and 95% for C1 and C2, respectively (Fig. 1A). Moreover, C1 was composed of three distinct sub-clusters with sub-C1 (intra-cluster SR=94%), sub-C2 (intra-cluster SR=97%), sub-C3 (intra-cluster SR=94%) with sub-C2 exclusively composed of outbreak strains. C2 cluster was composed of two sub-clusters, sub-C4 (intra-cluster SR=96%) and sub-C5 (intra-cluster SR=96%) (Fig. 1B). Most of the typed *A. baumannii* strains shared multidrug resistance phenotype with resistance to most antibiotics tested, apart from O2, NO2 and NO3 exhibiting sensitivity to aminoglycosides and NO9, NO11 and NO13 to trimethoprim/sulfamethoxazole (Fig. 1C).

**Funding**

B.B.X. was supported by University of Antwerp Research funds (BOF-DOCPRO 2012-27450). This work was financially supported by Opgen, Inc, USA in the frame of European Public Health Initiative (EUPHi) and Platform for European Preparedness Against (Re-)emerging Epidemics, EU-FP7 (PREPARE; 602525).

---