

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

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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 by 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 disc diffusion.

Conclusions

This study revealed high genomic heterogeneity of the typed *A. baumannii* clinical isolates (SR of $\geq 44\%$) 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 WGM.

References

1. Miller JM. Whole-Genome Mapping: a New Paradigm in Strain-Typing Technology. *Journal of Clinical Microbiology*. 2013;51(4):1066-1070. doi:10.1128/JCM.00093-13.

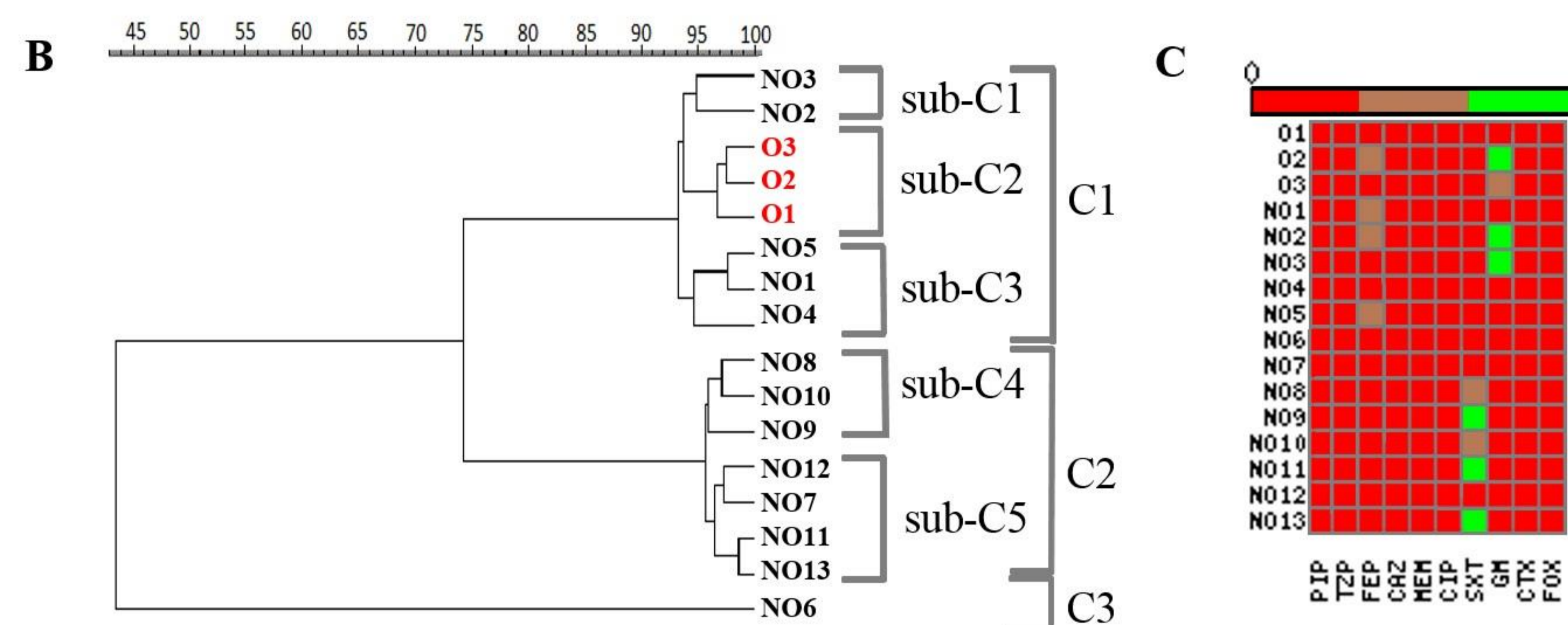


Figure 1: (B) Map similarity cluster generated from the whole genome maps. (C) Antibigrams of typed strains with resistance (in red) intermediate resistance (brown) and sensitivity (green) to (from left to right): piperacillin, piperacillin-tazobactam, cefepime, ceftazidime, meropenem, ciprofloxacin, trimethoprim-sulfamethoxazole, gentamycin, cefotaxime and ceftiofur.

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**).

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