Streamlined Genomic Approaches for Studying Virulence Markers in *Acinetobacter baumannii (Ab)*

E. Levy[1], N. Gilad[1], M. Gordon[1], D. Marchaim[2], J.A. Carrico[3], M. Ziv-Ukelson[1], V. Chalifa-Caspi[1] and J. Moran-Gilad[1,4,5]

[1]Ben-Gurion University of the Negev, Beer Sheva, Israel; [2]Assaf Harofeh Medical Center, Zerifin, Israel; [3]Instituto de Microbiologia, Instituto de Medicina Molecular, Fac Medicina, Univ Lisboa, Portugal.; [4]Public Health Services, Ministry of Health, Jerusalem, Israel; [5]ESCMID Study Group for Molecular Diagnostics

Introduction

- Ab is a therapeutic and infection control challenge worldwide.
- Despite research efforts, knowledge regarding genetic virulence determinants (virulome) underlying Ab infections and their outcome is limited.
- Additionally, virulence characteristics of successful Ab genetic lineages have not been extensively studied.
- We sought to study the virulence characteristics of a diverse sample of Ab using whole genome sequencing (WGS).

Methods

- The analysis included 615 genomes (595 public; 20 Israeli blood isolates recovered in a single hospital sequenced using Miseq (PE 250bp)).
- Classification to species and assignment into sequence type (Oxford scheme) and lineage (International clone II/CC92) were performed using an in house bioinformatics pipeline and Seqshpere+ (Ridom GmbH).
- Three virulence gene datasets were separately analysed: 327 Ab-specific genes obtained via literature search and 7,471 and 30,157 genes from VFDB and mVirDB databases, respectively.
- The protein sequences from each dataset were BLASTed against each of the genomes. Hits with e-value <1e-7 covering >90% of the query with at least 30 aa were retained.
- Clinical metadata were used to divide 20 Israeli genomes into outcome Groups: G1–death within 24h of diagnosis; G2–death within 2-7 days of diagnosis; G3–death >7d of diagnosis or survival.
- Subsets of genomes were compared based on proportion of present genes.
- In addition, clustering analyses were used to identify shared gene groups amongst subsets of samples.

Results

Fig. 1 Characteristics of samples

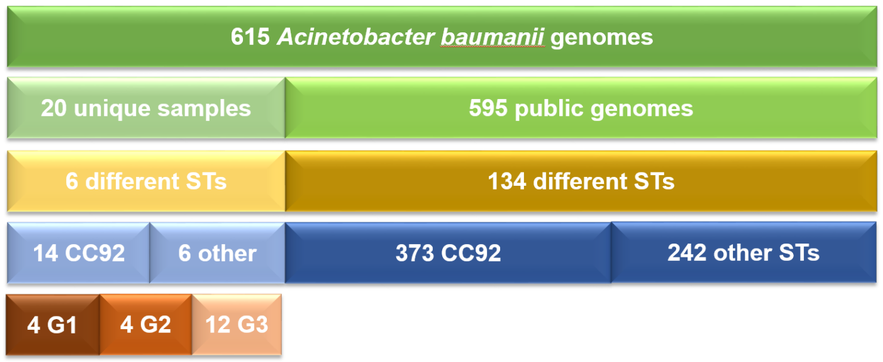


Fig. 2 MLST analysis of study samples

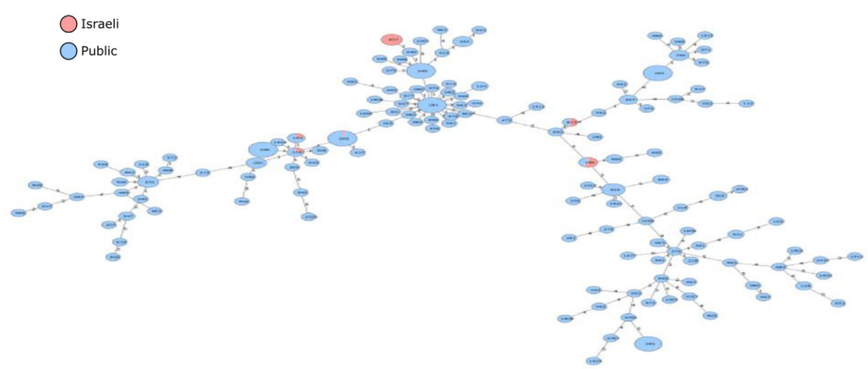


Fig. 3 Genes enriched in CC92 samples

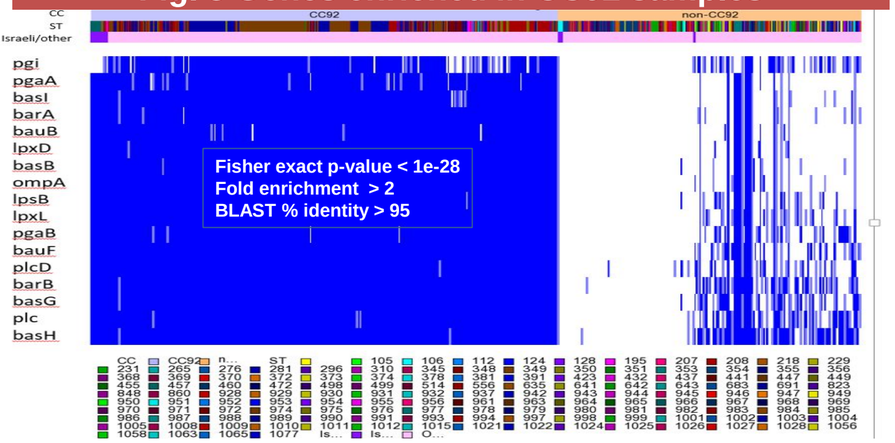


Fig. 4 Gene clustering in unique CC92 samples

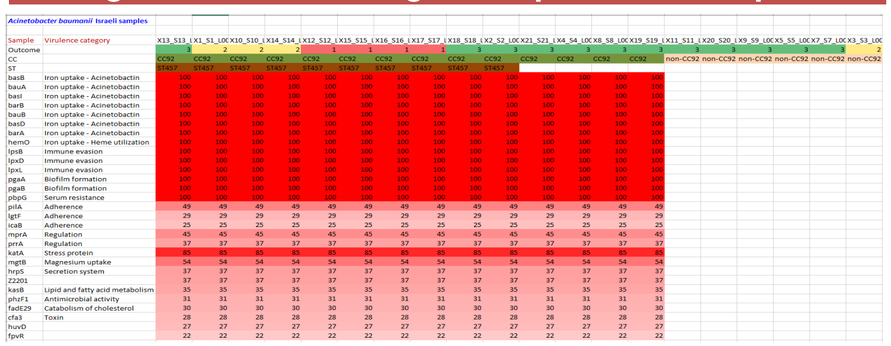
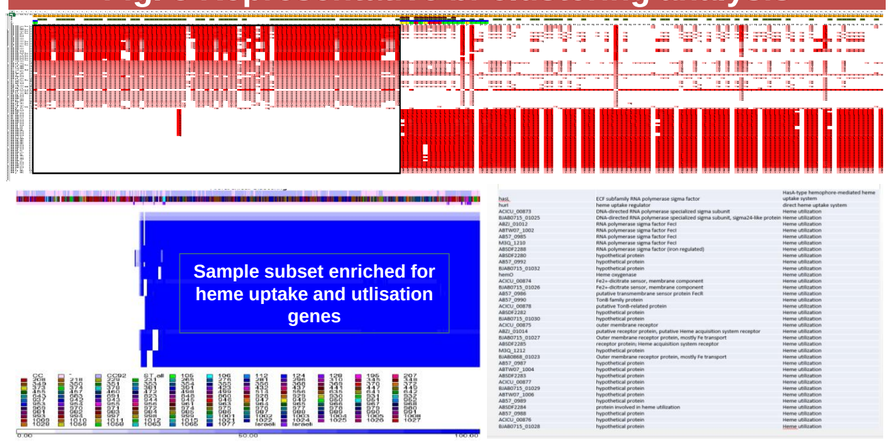


Fig. 5 Representative bi-clustering analysis



Conclusions

- Analysis of WGS data of Ab allowed a high-throughput fit-for-purpose screening across a diverse sample of strains, thus providing further insights on the virulome characteristics.
- CC92 genomes were enriched which genes related to iron uptake, immune evasion, biofilm formation and serum resistance.
- An attempt to correlate virulence genes to outcome based on a small patient subset by different methods did not identify virulence determinants clearly associated with an unfavourable outcome.
- Further study using larger clinical strain sets is thus warranted.