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Microbial pathogenesis reloaded

Streamlined genomic approaches for studying virulence markers in Acinetobacter baumannii (Ab)

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Background: 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).

Material/methods: The analysis included 615 Ab genomes (595 public and 20 Israeli samples of blood isolates recovered in a single hospital and subject to WGS 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 analysed: 327 Ab-specific genes obtained through 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

Results: The 615 genomes included 134 different STs and 373 (60.6%) belonged to CC92. Of 20 Israeli genomes, 4 each belonged to G1 and G2 and 12 to G3; 14 were CC92 and 10 were CC92/ST457. Amongst Israeli samples, 29 genes were present exclusively in CC92 genomes, 14 of which had 100% identity. The latter included 7 genes involved in acinetobactin-mediated iron uptake (basB, bauA, basI, barB, bauB, basD, barA), 3 in immune response evasion (lpsB, lpxD, lpxL) and the rest in heme utilisation, biofilm formation and serum resistance. No genes clearly distinguished between outcome groups. Considering the entire set of genomes, 17 genes were >2-fold more prevalent in CC92 compared to non-CC92 strains (Fisher exact test p-value < 1e-28; only genes with >95% identity in CC92 genomes included). Clustering analysis identified groups of genes highly enriched in certain strain groups (ST349/ST457, ST457, ST348/ST350, ST368, ST218)

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 of this species. 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 and thus further study using larger clinical strain sets is warranted.