RND efflux pumps across the *Acinetobacter* genus

Elizabeth M. Darby[1], Vassily N. Bavro[2], Steven Dunn[1], Alan McNally[1] & Jessica M. A. Blair[1].
[1]University of Birmingham, B15 2TT, [2]University of Essex, CO4 3SQ

Introduction

- *A. baumannii* is on the WHO priority pathogens list due to the number of drug resistant infections it causes.
- Other *Acinetobacter* species also cause human infections for example *A. lwoffii*, which is the leading cause of *Acinetobacter*-derived bacteraemia in England (PHE, 2019).
- *A. baumannii* is often multidrug resistant and this is mediated by acquired resistance genes, mutations in genes encoding the target of antibiotics and increased expression of multidrug efflux pumps.
- To date, nine RND genes have been characterised in *Acinetobacter* (mainly *A. baumannii)*: *adeJ, adeB, adeE, adeG, adeY, abeD, arpB, acrB and czcA* (Kornelsen *et al.*, 2021).

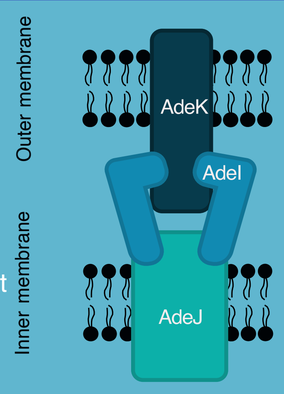

Figure 1: Schematic of RND system AdeIJK

- A method to search genomes for RND proteins was developed to find the number across the entire *Acinetobacter* genus.
- By mapping this data onto the phylogeny of the genus we show that efflux pumps annotated as AdeIJK and AdeXYZ are orthologous RND systems.
- **AdeIJK is ubiquitous,** found across all *Acinetobacter* species.
- We hypothesise that other RND systems, such as AdeABC may have been acquired in distinct disease-causing *Acinetobacter* species.

Materials and Methods

Highlighting total RND proteins in whole genome sequences

The consensus (>80%) amino acid sequence of characterised RND proteins was taken to create a conserved residue file, which was input into BLASTp to find RND proteins from *Acinetobacter* (NCBI, 2021). 4 reference sequences per *Acinetobacter* species were downloaded from NCBI (n=170).

Searching for known RND genes and infection-causing species

The reference sequences were searched using ABRicate (v.0.8.13) with a custom efflux database (Seeman, 2020a). ABRicate cut-off values of >50% identity and >50% coverage were used to highlight orthologous genes. A PubMed literature search determined if a given species had been documented to cause human infection.

Phylogenetic tree of *Acinetobacter*

A tree was created using a core gene alignment generated by Panaroo (v.1.2.3) as an input for Fasttree (v.2.1.10) (Tonkin-Hill *et al.*, 2020). Fasttree used a generalised time reversible model of evolution (Price *et al.,* 2010).

Genomic context and recombination of AdeIJK/AdeXYZ

10 Kb +/- *adeIJK* from *A. baumannii* AYE (CU459141.1), *A. lwoffii* 5867 (GCA_900444925.1) and *A. baylyi* ADP1 (CR543861.1), was visualised in Easyfig (v.2.2.5), with tBLASTx homology annotated (NCBI, 2021; Sullivan *et al.,* 2011). Furthermore, whole genome alignments of *A. baumannii* (n=100) were created using Snippy (4.6.0) and Gubbins (v.3.1.3) predicted recombination (Croucher *et al.,* 2015; Seeman, 2020b). Snippy and Gubbins data were presented using Phandango (Hadfield *et al.,* 2018).

Results

1. **AdeIJK is found in all *Acinetobacter* species** and species associated with infection often have additional RND systems, including AdeABC and AdeFGH.

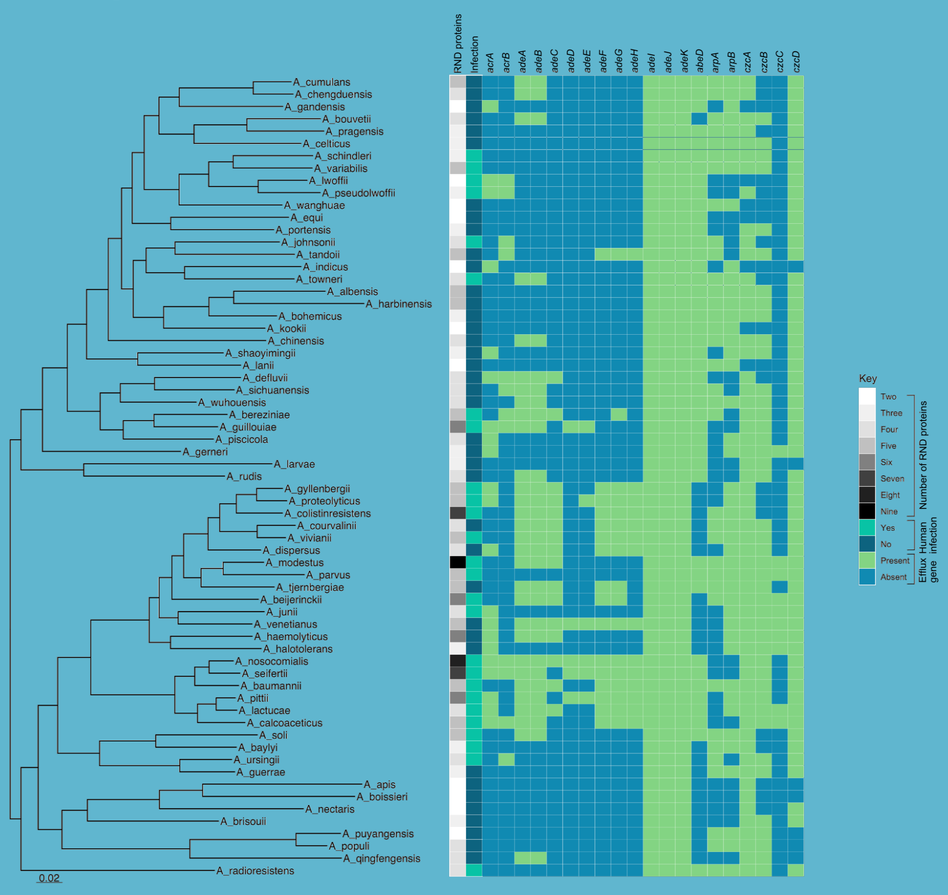

Figure 2: AdeIJK is the ancestral efflux pump in the Acinetobacter genus
Column 1- mean of the total number of (HME and HAE) RND proteins for each species, to 1 decimal place. The greater the number of proteins, the darker grey. Column 2 - if a species has been shown in the literature to cause infection it is turquoise. Columns 3-22 are green if the gene was present in the reference sequences

2. Using a **novel conserved RND residue BLASTp method**, it is possible to predict the number of HAE and HMD RND proteins in a given genome (column 2 of Fig.2)

3. **AdeIJK and AdeXYZ are orthologous RND systems**, they have a very **similar genomic context** (Fig. 3) and the **levels of recombination are** low around AdeIJK (Fig. 4).

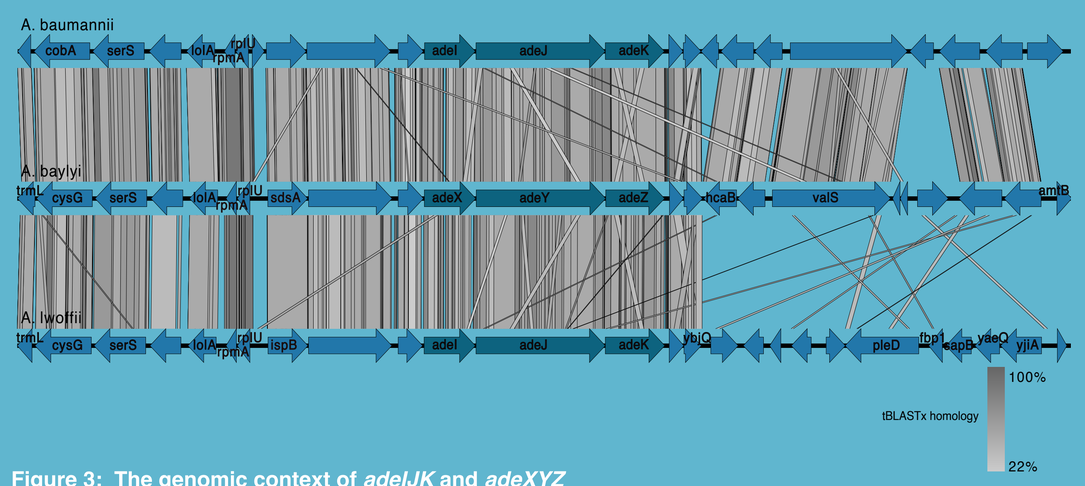

Figure 3: The genomic context of *adeIJK* and *adeXYZ*
Figure was created in Easyfig (v.2.2.5) using *A. baumannii* AYE, *A. baylyi* ADP1 and *A. lwoffii* 5867 sequences +/-10 Kb from the *ade* operons, annotated using Prokka (Seemann, 2014). The grey scale shows tBLASTx homology and blue arrows refer to coding regions within the genome. Immediately around *adeIJK/XYZ* is conserved, but differs further downstream of the OMF. *A. baumannii* and *A. baylyi* are more similar downstream of the OMF, compared to *A. lwoffii*.

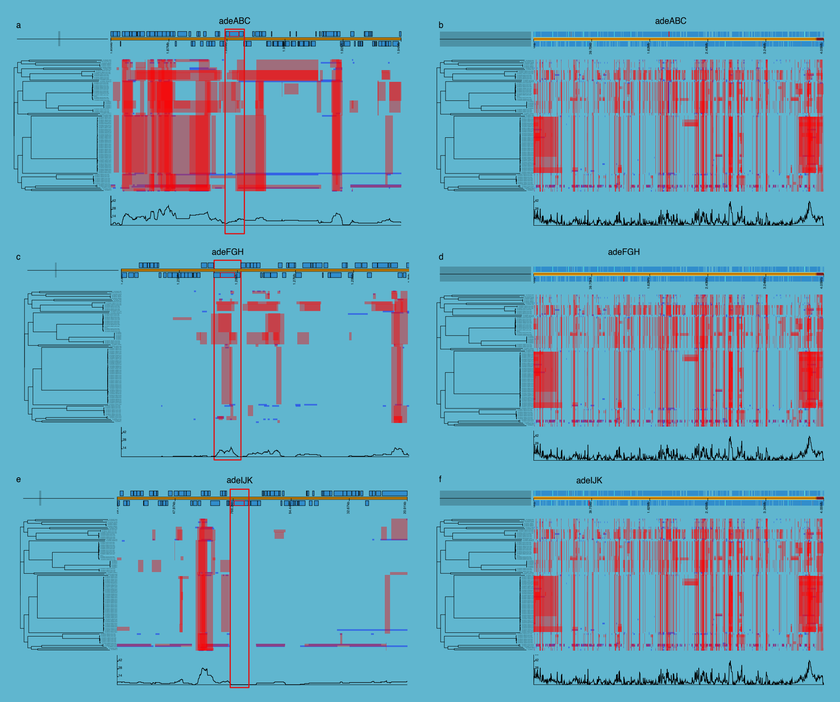

Figure 4: Recombination and SNPs in *ade* operons in 100 *A. baumannii* sequences
Parts a, c and e are zoomed in parts of the genome, showing the levels of SNPs (red and blue squares, red are ancestral SNPs) and recombination levels (the black line on the bottom). The right hand side, b, d and f, shows the entire genome and each time a different *ade* operon is highlighted. All figures have an associated phylogenetic tree created by Snippy to show the relatedness of the *A. baumannii* sequences.

Conclusions

- The number of RND proteins within whole genome sequences was predicted and validated in Gram negative species with well-characterised genomes (data not shown).
- AdeIJK and AdeXYZ are orthologous efflux systems that differ mainly in nomenclature.
- The lack of recombination and SNPs around AdeIJK indicate it is highly conserved.
- Further work to verify this phenotypically by assessing function of AdeXYZ from *A. baylyi* and AdeIJK from other *Acinetobacter* species is ongoing.
- AdeIJK is important for protecting against antibacterial host-associated fatty acids and modulating the bacterial cell membrane (Jiang *et al.,* 2019). Therefore, it is unsurprising that it is highly conserved within the *Acinetobacter* genus.

- Across *Acinetobacter*, more RND pumps are associated with infection-causing species
- Previous work has shown that *A. baumannii* over-expressing AdeABC was more virulent in the lungs of a mouse model (Yoon *et al.*, 2016).
- Infection-causing *Acinetobacter* species are often less susceptible to antibiotics than environmental *Acinetobacter* and this could also be, in part, due to the expression of additional efflux pumps. AdeABC overexpression is known to contribute to phenotypic antibiotic resistance whereas AdeIJK is linked to intrinsic resistance (Damier-Piolle *et al.,* 2008; Yoon *et al.,* 2013).

References

Croucher, N. *et al.,* (2015). Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Research*, *43*(3), e15–e15.
Damier-Piolle, L., *et al.,*(2008). AdeIJK, a resistance-nodulation-cell division pump effluxing multiple antibiotics in Acinetobacter baumannii. *Antimicrobial Agents and Chemotherapy*, *52*(2), 557–62.
Hadfield, J., *et al.,* (2018). Phandango: an interactive viewer for bacterial population genomics. *Bioinformatics*, *34*(2), 292–293.
Jiang, J.-H.,*et al.,* (2019). Identification of Novel Acinetobacter baumannii Host Fatty Acid Stress Adaptation Strategies. *MBio*, *10*(1).
Kornelsen, V., & Kumar, A. (2021). Multidrug Resistance Efflux Pumps in Acinetobacter spp.: An Update. *Antimicrobial Agents and Chemotherapy*.
NCBI. (2021). BLAST: Basic Local Alignment Search Tool. Retrieved April 3, 2021, from https://blast.ncbi.nlm.nih.gov/Blast.cgi
Price, M. N., *et al.,* (2010). FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE*, *5*(3), e9490.
PHE. (2019). *Laboratory surveillance of Acinetobacter spp. bacteraemia in England, Wales and Northern Ireland: 2018*.
Seeman, T. (2020a). ABRicate. Retrieved from https://github.com/tseemann/abricate
Seeman, T. (2020b). Snippy. Retrieved from https://github.com/tseemann/snippy
Sullivan, *et al.,* (2011). Easyfig: a genome comparison visualizer. *Bioinformatics*, *27*(7), 1009–1010. Tonkin-Hill, G., MacAlasdair, N., Ruis, C., Weimann, A., Horesh, G., Lees, J. A., … Parkhill, J. (2020). Producing polished prokaryotic pangenomes with the Panaroo pipeline. *Genome Biology*, *21*(1), 180.
Yoon, E.-J., *et al.,* (2016). Contribution of the Ade Resistance-Nodulation-Cell Division-Type Efflux Pumps to Fitness and Pathogenesis of Acinetobacter baumannii. *MBio*, *7*(3).
Yoon, E.-J., *et al.,* (2013). RND-type efflux pumps in multidrug-resistant clinical isolates of Acinetobacter baumannii: major role for AdeABC overexpression and AdeRS mutations. *Antimicrobial Agents and Chemotherapy*, *57*(7), 2989–95.

@lizzy_darby

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