

**British Society for  
Antimicrobial  
Chemotherapy**

**Antibiotic Resistance  
Mechanisms -  
Workshop for  
Researchers**

**8 – 9 November 2007**

**Holiday Inn,  
Birmingham Airport**

# Do large chromosomally integrated genomic islands contribute to resistance dissemination in *Acinetobacter* spp?

Farha Shaikh<sup>a</sup>, Richard P. Spence<sup>a,b</sup>, Katrina Levi<sup>c</sup>, Kevin. J. Towner<sup>c</sup>, Kumar Rajakumar<sup>a,b</sup>

<sup>a</sup> Dept of Infection, Immunity and Inflammation, University of Leicester, Leicester, UK

<sup>b</sup> Dept of Clinical Microbiology, Leicester Royal Infirmary, Leicester, UK

<sup>c</sup> Dept of Microbiology, University Hospital, Nottingham, UK

**BSAC**  
British Society for  
Antimicrobial Chemotherapy

## INTRODUCTION

*Acinetobacter baumannii* and other closely related *Acinetobacter* spp. are a major cause of nosocomial infections, particularly pneumonia in intensive care patients, leading to prolonged hospital and ICU stays. There are an increasing number of reports describing extensive antimicrobial resistance among *Acinetobacter* spp. and this has led to widespread recognition of the marked propensity of *Acinetobacter* to rapidly develop resistance to multiple classes of antimicrobial agents. This is a very worrying trait as it reduces the prospect of therapeutic success and ultimately poses the threat of a post-antibiotic era<sup>(1)</sup>. Despite extensive research into the pivotal role of integron- and transposon-mediated antibiotic resistance in the evolution of *Acinetobacter* resistance, very little is known about the larger genomic context within which these mobile resistance determinants lie.

**Aim:** To investigate the *ATPase* gene as a "hotspot" for integration of genomic islands in a range of *A. baumannii* strains; we further aimed to capture and characterize these integrated elements using a chromosome walking technique and a yeast-based recombinatorial system to elucidate the role in the virulence of these *A. baumannii* *ATPase*-integrated genomic islands.

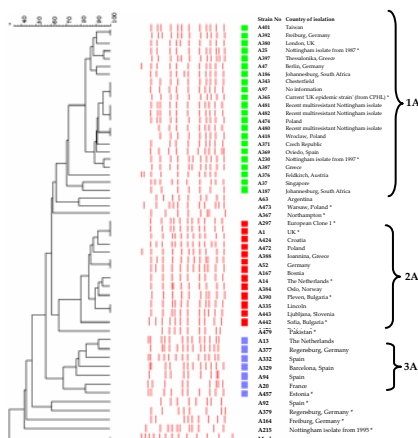
## HYPOTHESIS

In the early 1990s, Elisha and Steyn<sup>(2)</sup> reported a chromosomal resistance locus in a strain of *A. baumannii* that resembled a Tn2670-like transposon. Rajakumar *et al.* (1997) subsequently noticed a marked similarity between this *Acinetobacter* locus and a segment of the *Shigella* Resistance Locus (SRL) pathogenicity island that was believed to contribute to the virulence of *Shigella*. This led us to hypothesize that a proportion of chromosomal antibiotic resistance-encoding loci in *A. baumannii* and other related *Acinetobacter* spp. are borne on large chromosomally integrated resistance islands that themselves contain integron or transposon structures. The prevalence of class 1 integrons in *A. baumannii* further supported our hypothesis, since the SRL pathogenicity island itself harboured a typical class 1 integron. Indeed, the incidental discovery of an 86 kb resistance island in a single strain of *A. baumannii* (strain AYE)<sup>(3)</sup>, further substantiates our hypothesis.

**University of Leicester**

## METHODS & RESULTS

### Genotyping of *A. baumannii* isolates



**Dendrogram showing phylogenetic relationship between 50 *A. baumannii* isolates based on Random Amplified Polymorphic DNA (RAPD) analysis.**

- A total of 50 geographically diverse, multi-resistant *A. baumannii* strains were analyzed by RAPD-PCR typing, to determine their epidemiological relatedness.
- PCR typing with primer DAF3 resulted in 42 strains falling into 3 distinct RAPD patterns; 1A (n = 22), 2A (n = 13), 3A (n = 7). The remaining eight isolates that exhibited <70% pattern similarity were considered to be outliers.
- Representative isolates from each cluster (\*) were then selected for further analysis.

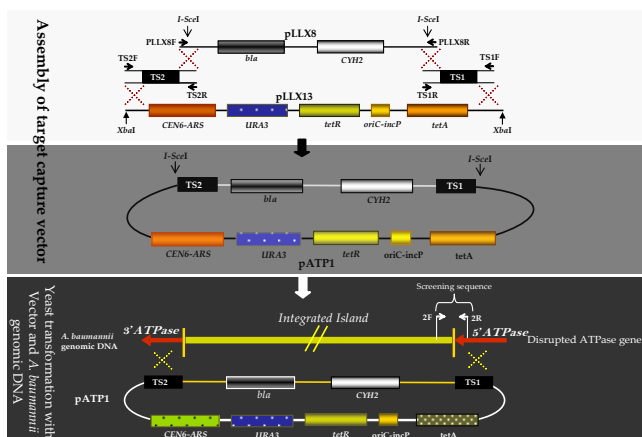
### Recombinational cloning of bacterial genomic DNA in yeast

**STRATEGY:** Based on the recent published report highlighting the *ATPase* gene as a potential "hotspots" for integration of genomic islands in *A. baumannii*, we constructed a yeast capture vector for recombinational cloning of genomic islands integrated into the *ATPase* gene in different *A. baumannii* strains. This approach has been previously described for capturing genomic islands in strains of *Pseudomonas aeruginosa*<sup>(4)</sup>.

### Construction of yeast capture vector

The capture vector was assembled from four DNA sequences by using recombinational cloning in the yeast *Saccharomyces cerevisiae*, where homologous recombination is the favored pathway for DNA double-strand break repair.

- *Xba*I-linearized pLLX13 vector backbone which carries a yeast selectable marker (*URA3*), plasmid maintenance elements *CEN6* and *ARSH4*, and the bacterial selectable tetracycline resistance marker (*tetAR*).
- PCR amplification of a 4.6kb fragment using primers (PLLX8F/R) from pLLX8, that carries a yeast cycloheximide counter-selectable gene (*CYH2*) and the  $\beta$ -lactamase gene *bla*, that confers carbenicillin resistance in *E. coli*.
- The two targeting sequences (TS1 & TS2) generated by PCR amplification using primers (TS1F/R & TS2F/R), corresponding to the 5' and 3' ends of disrupted *ATPase* gene. Inclusion of flanking homology on the four components facilitated the assembly of the capture vector by homologous recombination when co-transformed into competent *S. cerevisiae*.



### Investigation of the *ATPase* gene site in *A. baumannii* isolates for genomic islands (GI)

#### STRATEGY

- PCR analysis of fifty *A. baumannii* strains, with primer pair (2F/4R) corresponding to regions within the upstream (U) & downstream (D) flanks of the *ATPase* gene.
- Representative *A. baumannii* strains with a disrupted *ATPase* gene were further investigated by PCR amplification for the presence of: *tniA*, a transposase gene found at the 3' *ATPase*-end of AbaR1 (3F/4R) and *sulI*, a sulphate permease gene, present at the 5' *ATPase*-end of AbaR1 (2F/3R).



- *ATPase* PCR-*ve* strains were also investigated using a chromosome walking technique known as single genome-specific PCR (SGSP-PCR). This involves generating separate restriction libraries for each test strain, as a template for PCR. PCR amplification with the 2F or 4R genome-specific primer & a second universal vector primer (T7 or T3), produces amplicons that "walk" into the proximal or distal ends of island sequences

## CONCLUSIONS

- PCR interrogation of the *ATPase* gene, that harbours the integrated AbaR1 in *A. baumannii* strain AYE, in fifty other *A. baumannii* strains failed to produce an amplicon with forty-one strains, suggesting the presence of *ATPase*-integrated elements in these strains as well.
- Chromosome walking from both ends of the disrupted *ATPase* gene demonstrated the presence of AbaR1-like sequences within the *ATPase* genes in at least five out of six strains tested.
- PCR mapping using *tniA*- and *sulI*-specific primers produced amplicons of identical sizes to those predicted for AYE in three of fourteen for both *tniA* and *sulI* and a further three strains for *tniA* only, suggesting that the extremities of the islands integrated into these strains closely resembled AbaR1 in AYE.
- We have successfully constructed a yeast capture vector, pATP1, that will be applied to clone intact islands from a large number of *ATPase*-PCR negative *A. baumannii* strains.
- **Future work:** capture *ATPase*- islands in different *A. baumannii* strains, characterize in detail by sub-cloning/sequencing and analyse data to postulate roles in virulence and/or resistance accumulation.

## REFERENCES

1. *Acinetobacter* Outbreaks 1977-2000. Villegas MV, Hartstein AL. Infection control and hospital epidemiology. 2003.
2. Identification of an *Acinetobacter baumannii* gene region with sequence and organizational similarity to Tn2670. Elisha and Steyn, Plasmid 25: 96-101, 1991.
3. Comparative Genomics of Multidrug resistance in *Acinetobacter baumannii*. Pierre-Edouard Fournier, David Valletet, Valérie Barbe, Stéphanie Audic, Hironori Ogata, Laurent Poirat, Hervé Robert, Catherine Robert, Sophie Mangonot, Chantal Aberger, Patrice Nordmann, Jean Weissenbach, Didier Raoult, Jean-Michel Claverie. PLoS Genet 2(1):e7, 2006.
4. Conservation of genome content and virulence determinants among clinical and environmental isolates of *Pseudomonas aeruginosa*. Matthew. C. Wolfgang, Bridget. R. Kulkarni, Xiaoyu Liang, Dana Boyd, Kai Wu, Qing Yang, Garrett Miyada and Stephen Lory. PNAS 2005.

## ACKNOWLEDGEMENTS

The authors would like to thank the British Society for Antimicrobial Chemotherapy (BSAC) for funding this project.