

**FACTORS CONTRIBUTING TO  
THE SUCCESS OF  
*ACINETOBACTER BAUMANNII*  
AS A HUMAN PATHOGEN**

BY

BART A. EIJKELKAMP

**A thesis submitted for the Degree of Doctor of Philosophy**

**The School of Biological Sciences**

**Flinders University**

**October 2011**

# TABLE OF CONTENTS

<b>TABLE OF FIGURES .....</b>	<b>VII</b>
<b>DATA TABLES.....</b>	<b>IX</b>
<b>ABSTRACT .....</b>	<b>X</b>
<b>DECLARATION .....</b>	<b>XII</b>
<b>ACKNOWLEDGEMENTS.....</b>	<b>XIII</b>
<b>CONTRIBUTIONS.....</b>	<b>XIV</b>
<b>PUBLICATIONS.....</b>	<b>XV</b>
<b>CHAPTER 1 GENERAL INTRODUCTION .....</b>	<b>1</b>
1.1 <i>A. baumannii</i> ; a significant human pathogen with an alarming potential .....	2
1.1.1 <i>Acinetobacter</i> classification remains under debate .....	3
1.1.2 The global spread of successful <i>A. baumannii</i> clonal lineages.....	4
1.1.3 <i>A. baumannii</i> can cause a wide range of infections .....	5
1.1.4 Cost-related consequences in the clinical setting.....	5
1.1.5 <i>A. baumannii</i> as a community-acquired pathogen .....	6
1.1.5.1 Carriage of <i>Acinetobacter</i> .....	6
1.1.5.2 Prevalence of community-acquired <i>A. baumannii</i> is higher in (sub)tropical areas .....	6
1.1.5.3 Post-traumatic community-acquired <i>A. baumannii</i> infections .....	7
1.2 Mechanisms involved in <i>A. baumannii</i> virulence and persistence.....	7
1.2.1 Iron acquisition mechanisms.....	8
1.2.1.1 The importance of iron acquisition for pathogens.....	8
1.2.1.2 Siderophore-mediated iron acquisition .....	8
1.2.1.3 Iron acquisition in <i>A. baumannii</i> .....	9
1.2.2 Adherence to abiotic surfaces and biofilm formation.....	9
1.2.2.1 Persistence of <i>A. baumannii</i> in the hospital environment .....	9
1.2.2.2 Various mechanisms contribute to abiotic surface adherence and biofilm formation.....	11
1.2.3 The interaction between <i>A. baumannii</i> and eukaryotic cells.....	12
1.2.4 <i>Acinetobacter</i> motility characteristics.....	14
1.2.4.1 The different forms of bacterial motility.....	14
1.2.4.2 The role of motility in virulence.....	14
1.2.4.3 Motility of <i>Acinetobacter</i> species.....	15
1.3 <i>A. baumannii</i> contains a broad arsenal of resistance mechanisms .....	16

1.3.1	Antibiotic modifying enzymes .....	16
1.3.2	Resistance as a result of genetic mutations .....	17
1.3.3	Resistance due to loss of porin proteins .....	17
1.3.4	Efflux-mediated resistance .....	18
1.3.4.1	The ATP-binding cassette superfamily .....	20
1.3.4.2	The major facilitator superfamily .....	20
1.3.4.3	The small multidrug resistance family .....	21
1.3.4.4	The multidrug and toxic compound extrusion family .....	22
1.3.4.5	The resistance-nodulation-cell division superfamily .....	23
1.3.5	The need for development of novel antimicrobial therapies.....	24
1.3.5.1	‘Last resort’ antibiotics.....	25
1.3.5.2	Combination therapies.....	26
1.3.5.3	Prospective antimicrobial agents.....	26
1.3.5.4	Targeting virulence determinants.....	27
1.4	What have genomic analyses taught us so far? .....	28
1.4.1	AbaR-type resistance islands .....	29
1.4.2	<i>A. baumannii</i> plasmids are highly variable between strains .....	31
1.4.3	The role of insertion sequences in shaping the <i>A. baumannii</i> genome .....	31
1.5	Scope of this thesis .....	32
<b>CHAPTER 2 MATERIALS AND METHODS .....</b>		<b>34</b>
2.1	Buffers and growth media .....	35
2.1.1	Solutions and buffers .....	35
2.1.2	Bacterial culture media .....	35
2.2	Bacterial strains and growth conditions .....	35
2.3	Bacterial characterisation assays .....	45
2.3.1	International clone determination .....	45
2.3.2	Minimal inhibitory concentration assays .....	45
2.3.3	Static biofilm formation assays .....	46
2.3.4	Motility assays .....	46
2.3.5	Pellicle formation assays.....	47
2.3.6	Eukaryotic cell adherence assays.....	47
2.3.7	Hydrophobicity test.....	48
2.3.8	Phenotype MicroArray analysis.....	48
2.3.9	Statistical analyses .....	48
2.4	DNA and RNA techniques .....	48
2.4.1	Plasmid DNA isolation .....	48
2.4.2	Genomic DNA isolation.....	49
2.4.3	RNA isolation .....	49
2.4.4	DNA agarose gel electrophoresis.....	49

2.4.5	Purification of DNA fragments .....	50
2.4.6	Quantitation and quality assessment .....	50
2.4.7	DNA sequencing .....	50
2.4.8	Conventional cloning .....	50
2.4.9	Gateway cloning.....	51
2.4.10	Preparation of chemically competent <i>E. coli</i> cells.....	51
2.4.11	Transformation of chemically competent <i>E. coli</i> cells.....	51
2.4.12	Preparation of electrocompetent <i>A. baumannii</i> cells.....	52
2.4.13	Transformation of electrocompetent <i>A. baumannii</i> cells .....	52
2.4.14	Polymerase chain reaction.....	52
2.4.15	Quantitative reverse transcription-PCR .....	60
2.5	Microarray analyses .....	60
2.5.1	Array development.....	60
2.5.2	cDNA synthesis and microarray hybridisation .....	60
2.6	Protein detection.....	61
2.6.1	Sample preparation.....	61
2.6.2	DC-BCA protein assay.....	61
2.6.3	SDS-PAGE.....	62
2.6.4	Coomassie stain.....	62
2.6.5	Protein transfer to polyvinylidene fluoride membrane.....	62
2.6.6	Immunological detection of recombinant proteins .....	62
2.7	Bioinformatic analyses .....	63
2.7.1	Alignments and <i>in silico</i> manipulations.....	63
2.7.2	Genomic DNA analyses .....	63
2.7.3	Motif and DNA binding site analyses .....	64
<b>CHAPTER 3 INVESTIGATION OF THE HUMAN PATHOGEN</b>		
<b><i>ACINETOBACTER BAUMANNII</i> UNDER IRON-</b>		
<b>LIMITING CONDITIONS..... 65</b>		
3.1	Introduction .....	66
3.2	Results and Discussion.....	67
3.2.1	Optimisation of test conditions for transcriptomic analysis.....	67
3.2.2	Global transcriptional changes of <i>A. baumannii</i> ATCC 17978 to iron starvation.....	69
3.2.3	<i>A. baumannii</i> FUR box optimisation .....	73
3.2.4	Transcriptional profiling of the siderophore-mediated iron acquisition mechanisms .....	77
3.2.5	Investigation of motility under iron-limiting conditions.....	80
3.2.6	Comparative analysis of the iron acquisition mechanisms of sequenced <i>Acinetobacter</i> isolates.....	84
3.2.7	A second FUR-like transcription repressor.....	91
3.3	Conclusions .....	91

<b>CHAPTER 4 ADHERENCE AND MOTILITY CHARACTERISTICS OF CLINICAL <i>ACINETOBACTER BAUMANNII</i> ISOLATES .....</b>	<b>93</b>
4.1 Introduction .....	94
4.2 Results and Discussion.....	95
4.2.1 Strain selection and clonality .....	95
4.2.2 Motility of <i>A. baumannii</i> .....	95
4.2.3 Adherence to abiotic surfaces and biofilm formation .....	98
4.2.4 Adherence to eukaryotic cell surfaces.....	99
4.2.5 Genomic analysis of <i>A. baumannii</i> motility and adherence features .....	99
4.3 Conclusions .....	104
<b>CHAPTER 5 PHENOTYPIC AND MOLECULAR EXAMINATION OF A HYPER-MOTILE <i>ACINETOBACTER BAUMANNII</i> VARIANT STRAIN.....</b>	<b>107</b>
5.1 Introduction .....	108
5.2 Results and Discussion.....	109
5.2.1 Isolation of <i>A. baumannii</i> 17978hm; a hyper-motile variant strain ....	109
5.2.1.1 Motility characteristics .....	109
5.2.1.2 Stability of the variant strain .....	112
5.2.2 Adherence characteristics.....	114
5.2.2.1 Adherence to abiotic surfaces and biofilm formation .....	114
5.2.2.2 Pellicle formation .....	114
5.2.2.3 Adherence to eukaryotic cells .....	115
5.2.3 Cell surface hydrophobicity .....	117
5.2.4 Transcriptomic analysis of the motile versus non-motile population.....	120
5.2.4.1 Design of the study.....	120
5.2.4.2 Global transcriptional differences between motile and non-motile cells .....	121
5.2.5 Examination of metabolic differences .....	124
5.2.5.1 Expression levels of <i>paaA</i> .....	124
5.2.5.2 Carbon source utilisation.....	125
5.2.6 Signal transduction in <i>A. baumannii</i> .....	128
5.2.6.1 The genomic organisation of the region harbouring the quorum-sensing signal biosynthesis cluster .....	129
5.2.6.2 Investigation of AbaR binding sites .....	131

5.2.6.3	Lon protease .....	132
5.2.7	Genome sequence analysis of <i>A. baumannii</i> strains ATCC 17978 and 17978hm.....	133
5.2.7.1	Single nucleotide polymorphisms .....	133
5.2.7.2	Insertional inactivation of a gene encoding a putative histone-like protein.....	134
5.2.7.3	Complementation assays .....	140
5.2.8	The effect of stress on motility and adherence.....	142
5.2.8.1	The variant strain does not display differences in resistance .....	142
5.2.8.2	Inhibition of motility as a result of environmental stress .....	144
5.2.8.3	Stress on biofilm formation.....	148
5.2.8.4	Pellicle formation is susceptible to environmental changes.....	150
5.2.9	Transcriptional profiling of <i>A. baumannii</i> under stress conditions.....	152
5.2.9.1	Expression of <i>abaI</i> correlates with the motility phenotype.....	152
5.2.9.2	Expression of type IV pili is highly responsive to iron limitation .....	153
5.2.9.3	The phenylacetic acid degradation pathway is highly up-regulated in a saline environment.....	155
5.3	Conclusions .....	155

**CHAPTER 6 DEVELOPMENT OF A HIGH-THROUGHPUT CLONING STRATEGY FOR CHARACTERISATION OF ACINETOBACTER BAUMANNII DRUG TRANSPORTER PROTEINS..... 159**

6.1	Introduction .....	160
6.2	Results and Discussion.....	161
6.2.1	The prevalence of drug transporters in clinical <i>A. baumannii</i> isolates.....	161
6.2.2	Annotation and isolation of the <i>A. baumannii</i> efflux systems .....	165
6.2.3	Construction of a Gateway-based cloning system for heterologous expression of membrane proteins.....	166
6.2.4	Functional characterisation of <i>A. baumannii</i> efflux systems .....	169
6.2.5	Constructing directed <i>A. baumannii</i> knockout strains .....	172
6.3	Conclusions .....	176

**CHAPTER 7 CONCLUSIONS AND DISCUSSION..... 177**

7.1	<i>A. baumannii</i> persistence mechanisms.....	178
7.1.1	Biofilm formation .....	179
7.1.2	Pellicle formation.....	179
7.1.3	Interaction with eukaryotic cells .....	181

7.2	The mechanisms involved in <i>A. baumannii</i> motility.....	182
7.2.1	Pili-mediated motility .....	182
7.2.2	Hydrophobicity and biosurfactant production .....	183
7.2.3	Distinct <i>A. baumannii</i> strains employ different mechanisms for motility .....	185
7.3	Regulatory mechanisms involved in <i>A. baumannii</i> persistence.....	187
7.3.1	Quorum-sensing is essential for <i>A. baumannii</i> motility.....	187
7.3.2	The involvement of H-NS in expression of the <i>A. baumannii</i> persistence features .....	188
7.4	The importance of iron acquisition in <i>A. baumannii</i> .....	190
7.5	Efflux-mediated resistance .....	191
7.6	Genome analyses.....	193
7.7	Conclusions .....	195
<b>CHAPTER 8 APPENDICES.....</b>		<b>196</b>
Appendix A	Genes significantly up-regulated in <i>A. baumannii</i> strain ATCC 17978 under iron-limiting conditions .....	197
Appendix B	Genes significantly down-regulated in <i>A. baumannii</i> strain ATCC 17978 under iron-limiting conditions .....	209
Appendix C	ATCC 17978 FUR binding sites.....	227
Appendix D	Genes more than 2-fold up-regulated in <i>A. baumannii</i> strain 17978hm compared to strain ATCC 17978.....	230
Appendix E	Genes more than 2-fold down-regulated in <i>A. baumannii</i> strain 17978hm compared to strain ATCC 17978.....	236
Appendix F	Eijkelkamp <i>et al.</i> 2011; BMC Genomics.....	246
Appendix G	Eijkelkamp <i>et al.</i> 2011; FEMS Microbiology Letters .....	260
Appendix H	Eijkelkamp <i>et al.</i> 2011; Journal of Molecular Microbiology and Biotechnology .....	268
Appendix I	Abbreviations.....	277
<b>CHAPTER 9 REFERENCES .....</b>		<b>280</b>

## TABLE OF FIGURES

Figure 1.1: The five families of drug transporters.....	19
Figure 1.2: Genomic comparison of the <i>A. baumannii</i> AbaR resistance islands ....	30
Figure 3.1: Growth curves of <i>A. baumannii</i> strain ATCC 17978 with varying iron concentrations .....	68
Figure 3.2: Overview of transcriptional responses of <i>A. baumannii</i> strain ATCC 17978 to iron starvation.....	70
Figure 3.3: Microarray results of <i>A. baumannii</i> strain ATCC 17978 under iron-limiting and iron-replete conditions displayed by COG function .....	72
Figure 3.4: The optimised <i>A. baumannii</i> ATCC 17978 FUR motif.....	76
Figure 3.5: Transcriptional profiling of three siderophore gene clusters identified in <i>A. baumannii</i> strain ATCC 17978 .....	78
Figure 3.6: <i>A. baumannii</i> ATCC 17978 gene clusters with a putative role in motility .....	83
Figure 3.7: Swarming motility of <i>A. baumannii</i> strain ATCC 17978 .....	85
Figure 3.8: Comparison of siderophore cluster 1 between <i>A. baumannii</i> ATCC 17978 and SDF.....	88
Figure 4.1: Characterisation of <i>Acinetobacter</i> isolates.....	97
Figure 4.2: Adherence of <i>A. baumannii</i> strains to eukaryotic epithelial cells.....	100
Figure 4.3: Pila similarity analysis .....	102
Figure 5.1: Identification of the hyper-motile variant strain .....	110
Figure 5.2: Growth curves of <i>A. baumannii</i> ATCC 17978 and the hyper-motile variant.....	111
Figure 5.3: Analysis of the protein content of <i>A. baumannii</i> strain ATCC 17978 and the hyper-motile variant .....	113
Figure 5.4: Pellicle formation by <i>A. baumannii</i> strains ATCC 17978 and 17978hm.....	116
Figure 5.5: Adherence to A549 human lung epithelial cells .....	118
Figure 5.6: Cell surface hydrophobicity .....	119
Figure 5.7: Overview of transcriptional differences between motile and non-motile <i>A. baumannii</i> cells.....	122
Figure 5.8: Transcriptomic data of motile versus non-motile <i>A. baumannii</i> cells represented by COG function.....	123
Figure 5.9: Comparative analysis of carbon source utilisation.....	127



Figure 5.10: Genetic organisation of <i>A. baumannii</i> QS-regulated genes .....	130
Figure 5.11: Positioning of the insertion elements identified in A1S_0268 of <i>A. baumannii</i> strains 17978hm and B23 .....	137
Figure 5.12: Comparative analysis of the transcriptome results and genomic conservation .....	141
Figure 5.13: Motility phenotypes of <i>A. baumannii</i> strain 17978hm under stress ...	146
Figure 5.14: Biofilm formation under stress conditions.....	149
Figure 5.15: Pellicle formation of <i>A. baumannii</i> strain 17978hm under stress conditions .....	151
Figure 6.1: The Gateway-based expression systems used for expression of <i>A. baumannii</i> efflux proteins.....	168
Figure 6.2: Western blot detection of heterologously expressed <i>A. baumannii</i> efflux proteins in <i>E. coli</i> .....	170
Figure 6.3: The <i>A. baumannii</i> insertion disruption strategy developed in this study .....	174

## DATA TABLES

Table 2.1: Buffers and solutions .....	36
Table 2.2: Bacterial strains used in this study .....	37
Table 2.3: Plasmids used in this study.....	41
Table 2.4: Oligonucleotides used in this study .....	53
Table 3.1: Validation of the transcriptomic data by comparison of expression levels determined by microarray and qRT-PCR analysis.....	71
Table 3.2: Putative FUR binding sequences in the <i>A. baumannii</i> ATCC 17978 genome used for generating the FUR box motif .....	75
Table 3.3: Characteristics of the putative <i>A. baumannii</i> ATCC 17978 siderophore receptors.....	81
Table 3.4: Genomic comparison of siderophore gene clusters in sequenced <i>Acinetobacter</i> isolates.....	87
Table 3.5: The ability of different <i>Acinetobacter</i> strains to grow under iron-limiting conditions.....	90
Table 4.1: Presence of type I pili cluster in fully sequenced <i>A. baumannii</i> isolates .....	103
Table 5.1: Single nucleotide polymorphisms identified in the <i>A. baumannii</i> 17978hm genome .....	135
Table 5.2: Signal strengths of A1S_0268 and A1S_0628 in <i>A. baumannii</i> strains ATCC 17978 and 17978hm determined by microarray.....	139
Table 5.3: Complementation of the <i>A. baumannii</i> ATCC 17978 A1S_0268 mutant strains.....	143
Table 5.4: Antimicrobial MIC values of <i>A. baumannii</i> strain ATCC 17978 and 17978hm .....	145
Table 5.5: Motility of <i>A. baumannii</i> strain 17978hm under stress .....	147
Table 5.6: Transcriptional responses of <i>A. baumannii</i> strain 17978hm under different stress conditions.....	154
Table 6.1: Characteristics and prevalence of the <i>A. baumannii</i> ATCC 17978 drug transporters.....	162
Table 6.2: Substrate profile of heterologously expressed <i>A. baumannii</i> efflux proteins in <i>E. coli</i> .....	171

## ABSTRACT

*Acinetobacter baumannii* is a major problem in the hospital setting and also shows significance as a community-acquired pathogen in tropical climates, including parts of Australia. The increase in resistance to widely used antibiotics is evident and signs of pan-resistance are emerging. Epidemiological studies have shown global dissemination of successful clones, explaining the occurrence of troublesome *A. baumannii* outbreaks worldwide. However, the wide variety of *A. baumannii* resistance and persistence mechanisms remains poorly understood. This can be partially attributed to the phenotypic and genetic variation observed between clinical *A. baumannii* strains.

Iron acquisition systems are important virulence factors in pathogenic bacteria. To identify these systems in *A. baumannii*, the transcriptomic response of the fully sequenced strain ATCC 17978 under iron-limiting conditions was investigated. Of particular significance, three siderophore biosynthesis gene clusters, including one novel cluster, were highly up-regulated. Various genes involved in motility featured prominently amongst the genes down-regulated when iron was less readily available. Motility assays confirmed that these transcriptional changes are manifested at the phenotypic level.

The clonal relationship, and the motility and adherence characteristics of 54 Australian clinical isolates and various fully sequenced *Acinetobacter* strains were investigated. The majority of the strains were classified as part of the international clone groups I and II, as seen in other parts of the world. However, unlike distribution of clinical *A. baumannii* isolates in Europe or the United States, international clone III isolates were not identified in our collection. Motility was found to be a common trait in *A. baumannii* international clone I strains and in abundant biofilm formers not part of the international clone I group. A high level of variability in adherence to both abiotic surfaces and human lung epithelial cells was found.

A derivative of strain *A. baumannii* ATCC 17978, isolated in this study, was found to possess enhanced motility and adherence characteristics. An insertion event of a mobile genetic element into a gene encoding a histone-like protein (A1S\_0268)

was identified by whole genome sequencing. Introduction of the wild-type A1S\_0268 gene in the variant strain using an *Acinetobacter/E. coli* shuttle vector complemented the altered motility and adherence characteristics, making it indistinguishable to the wild-type *A. baumannii* ATCC 17978 parent. Transcriptional profiling of the variant strain under motile conditions assisted in identification of molecular mechanisms that play a putative role in *A. baumannii* adherence and motility. Investigation of motility, adherence and transcription levels of various molecular mechanisms, including type IV pili, under different conditions, such as low iron and high salt, showed that *A. baumannii* is highly responsive to stress.

Active transport of antimicrobial agents mediated by efflux proteins contributes to the high level of multidrug resistance observed in *A. baumannii*. Novel multidrug efflux systems were identified using a number of Gateway-based destination vectors constructed in this study. Additionally, a Gateway-based suicide vector was designed for construction of specific *A. baumannii* insertion disruption mutants. This knockout strategy was shown to be successful in disrupting a novel drug transporter.

Examination of the extensive collection of *A. baumannii* isolates at a genetic, transcriptional, protein and cellular level assisted in delineating factors that contribute to the success of *A. baumannii* as a human pathogen. The importance of various molecular mechanisms in persistence, resistance and disease potential has been described in this thesis.

# DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Bart A. Eijkelkamp

# ACKNOWLEDGEMENTS

I would first like to thank my supervisor Associate Professor Melissa Brown for giving me the opportunity to conduct my PhD project in her lab. Your support and advice throughout the last few years allowed me to get to this stage. There have been many people that have made working in the lab an enjoyable experience; Angela, Ming, Kim, Michael, Joanna, Sylvia, Eleni, Alisha, Mohsen, Charlotte, Sarah and Daniela, thanks guys, I could not have done it without you! A special thanks to Uwe, the lengthy discussions and casual chats were crucial for the project and for completing this thesis.

I would also like to acknowledge my co-supervisor Professor Ian Paulsen and the people in the Paulsen lab that I had the pleasure of working with; Sasha, Liam, Amanda, Kent, Ani and Dan, I am grateful for the numerous times you welcomed me in the lab in Sydney. Furthermore, I learned how to approach the project in a completely different way. Karl, I really enjoyed working with you and I will never forget your endless support. Even although it was on a distance, you have also become a real mate.

I am thankful to my friends on the third floor that introduced me to the great Australian lifestyle from the day I arrived; Nick, Emma, Pradeep, Simon, Bianca, Mel S, Patrick, Mel P, Ana, Drew, Peter, Chevaun and Lexi. The person that has kept me sane during my PhD is my girlfriend. Mel, you have always pushed me into having a life beside work. Being overseas for this long would not have been possible without you. Also, Ron and Jane thank you so much for everything!

Pa, ma en de rest van de familie, heel erg bedankt voor jullie steun, niet alleen voor de periode hier in Australië, maar voor de gehele studietijd. Ook wil ik mijn vrienden bedanken (met name Ruudje). Ookal is het lastig om over een grote afstand over de frustraties en successen van een promotie onderzoek te praten, ik weet dat jullie altijd achter me stonden. Ik hoop dat we in de nabije toekomst dichterbij elkaar kunnen wonen om weer eens ‘gewoon’ een biertje te kunnen drinken.

## CONTRIBUTIONS

Dr. KA Hassan (Macquarie University, Sydney, Australia) designed the microarray containing DNA probes to all predicted open reading frames of the *A. baumannii* ATCC 17978 genome and assisted with processing of the transcriptomic data. Furthermore, examination of carbon source utilisation by strains *A. baumannii* ATCC 17978 and a hyper-motile derivative of strain ATCC 17978, called *A. baumannii* 17978hm, were performed by Dr. KA Hassan using MicroPlate™ PM1 and PM2A (BIOLOG). The DNA sequence reads obtained by whole genome shotgun analysis of *A. baumannii* strains WM99c, D1279779, ATCC 17978 and 17978hm were assembled by Dr. LDH Elbourne (Macquarie University, Sydney, Australia). Bioinformatic identification of genes encoding putative drug transporters in the *A. baumannii* ATCC 17978 genome was also performed by Dr. LDH Elbourne, using the transautomated annotation pipeline (TransAAP; [www.membranetransport.org](http://www.membranetransport.org)). Part of the international clone determination PCRs were performed by Mr. MS Papadimitriou (Flinders University, Adelaide, Australia). The eukaryotic cell binding assays were performed in parallel with Dr. UH Stroeder (Flinders University, Adelaide, Australia).

# PUBLICATIONS

## Published work arising from data compiled in this thesis

**Eijkelkamp BA, Hassan KA, Paulsen IT and Brown MH** (2011). Investigation of the human pathogen *Acinetobacter baumannii* under iron-limiting conditions. *BMC Genomics*, **12**:126. (Appendix F).

**Eijkelkamp BA, Stroehler UH, Hassan KA, Papadimitriou MS, Paulsen IT and Brown MH** (2011). Adherence and motility characteristics of clinical *Acinetobacter baumannii* isolates. *FEMS Microbiology Letters*, **323**:44-51. (Appendix G).

**Eijkelkamp BA, Hassan KA, Paulsen IT and Brown MH** (2011). Development of a high-throughput cloning strategy for characterisation of *Acinetobacter baumannii* drug transporter proteins. *Journal of Molecular Microbiology and Biotechnology*, **20**:211-219. (Appendix H).

**Eijkelkamp BA, Hassan KA, Paulsen IT and Brown MH**. Efflux systems of the nosocomial pathogens *Staphylococcus aureus* and *Acinetobacter baumannii*. *Microbial Efflux Pumps: Current Research*. Manuscript submitted.

**Eijkelkamp BA, Stroehler UH, Hassan KA, Elbourne LDL, Paulsen IT and Brown MH**. H-NS plays a role in expression of the *Acinetobacter baumannii* persistence and virulence features. Manuscript in preparation.



## Abstracts

**Hassan KA, Penesyan A, Li L, Varkey D, Farrugia D, Eijkelkamp BA, Brown MH and Paulsen IT** Efflux mediated drug resistance in *Acinetobacter baumannii*. *ASM 2011*, Hotel Grand Chancellor, Hobart, Tasmania, Australia 4<sup>th</sup> – 8<sup>th</sup> July 2011.

**Hassan KA, Brzoska AJ, Wilson NL, Varkey DR, Eijkelkamp BA, Brown MH and Paulsen IT** Analysis of DHA2 family exporters in *Acinetobacter* spp. reveals putative functions in drug resistance and iron homeostasis, *Gordon Research Conferences on Multi-Drug Efflux Systems*, Les Diablerets Conference Center, Les Diablerets, Switzerland 12<sup>th</sup> – 17<sup>th</sup> July 2011.

**Eijkelkamp BA, Hassan KA, Paulsen IT and Brown MH** Investigation of the human pathogen *Acinetobacter baumannii* under iron-limiting conditions. 8<sup>th</sup> *International Symposium on the Biology of Acinetobacter*, University of Roma Tre, Roma, Italy 1<sup>st</sup> – 3<sup>rd</sup> September 2010.

**Eijkelkamp BA, Hassan KA, Paulsen IT and Brown MH** Investigation of the human pathogen *Acinetobacter baumannii* under iron-limiting conditions. *ASM 2010*, Sydney Convention Centre, New South Wales, Australia 4<sup>th</sup> – 8<sup>th</sup> July 2010.

**Eijkelkamp BA, Hassan KA, Papadimitriou MS, Cyza J, Paulsen IT and Brown MH** Characterisation of the *Acinetobacter baumannii* efflux systems. *BacPath10*, Novotel Barossa Valley Resort, South Australia, Australia 20<sup>th</sup> – 23<sup>rd</sup> September 2009.

**Eijkelkamp BA, Hassan KA, Paulsen IT and Brown MH** MATE pumps in the emerging pathogen *Acinetobacter baumannii*. *ASM 2009*, Perth Convention Exhibition Centre, Western Australia, Australia 6<sup>th</sup> – 10<sup>th</sup> July 2009.

**Hassan KA, Lim K, Eijkelkamp BA, Tetu SG, Ren Q, Elbourne LDH, Shaffer B, Loper JE, Brown MH and Paulsen IT** High-throughput functional genomics of bacterial efflux proteins. *Gordon Research Conference into Multidrug Efflux Systems*, Hotel Galvez, Galveston, Texas, USA 22<sup>nd</sup> – 27<sup>th</sup> March 2009.

**Eijkelkamp BA, Hassan KA, Paulsen IT and Brown MH** MATE pumps in the emerging pathogen *Acinetobacter baumannii*. *Gordon Research Conference into Multidrug Efflux Systems*, Hotel Galvez, Galveston, Texas, USA 22<sup>nd</sup> – 27<sup>th</sup> March 2009.

The presenting author has been underlined.