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

BY

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A thesis submitted for the Degree of Doctor of Philosophy

The School of Biological Sciences

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October 2011

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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 aftstand 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 dichter bij 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). Part of the international clone determination PCRs were performed by Mr. MS Papadimitrious (Flinders University, Adelaide, Australia). The eukaryotic cell binding assays were performed in parallel with Dr. UH Stroeher (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, Stroeher UH, Hassan KA, Papadimitrious MS, Paulsen IT and Brown MH (2011). Adherence and motility characteristics of clinical *Acinetobacter baumannii* isolates. *FEMS Microbiology Letters*, **323**:44-51. (Appendix G).

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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, Stroeher 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.

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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 12th – 17th July 2011.

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The presenting author has been underlined.