UNIVERSITI TEKNOLOGI MARA

GENOMIC ADAPTATION IN ANTIMICROBIAL RESISTANCE: ELUCIDATING THE ROUTE AND EFFECTS IN *Acinetobacter baumannii*

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Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Pharmacy

January 2016

CONFIRMATION BY PANEL OF EXAMINERS

I certify that a panel of examiners has met on 12th October 2015 to conduct the final examination of Mohamad Izwan Bin Ismail on his Doctor of Philosophy thesis entitled "Genomic Adaptation in Antimicrobial Resistance: Elucidating the Route and Effects in *Acinetobacter baumannii*" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated of knowledge as reference work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Postgraduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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Faculty Thesis Title	:	Genomic Adaptation in Antimicrobial Resistance: Elucidating the Route and Effects in *Acinetobacter baumannii*
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ABSTRACT

Antimicrobial resistance has been a looming threat ever since its conception and it has become one of the greatest global problems of the current era. Although various studies have been conducted to better understand the mutational triggers leading to antimicrobial resistance, the specific genomic path towards it have yet to be discerned. Here, we aim to elucidate the pathway of genomic evolution throughout the resistance induction of an *A. baumannii* strain towards ciprofloxacin, erythromycin, meropenem and imipenem, as well as comparing the mutations acquired clinically versus *in vitro*. Twenty-five (25) local clinical *A. baumannii* strains were isolated and screened for antimicrobial susceptibility, and their genome were sequenced using the Illumina GAIIx genome sequencer. The susceptible parent was then challenged with ciprofloxacin, erythromycin, meropenem and imipenem separately until growth is still possible beyond the Minimum Inhibitory Concentration (MIC) as defined by EUCAST standards. Once the resistance stability was confirmed, another sequencing run was done on the isogenic. Variant analysis was carried out using CLC Bio, and primers were designed to target the mutations of interest. PCR was then carried out on aliquots of the resistant mutants, each taken at increasing levels of antimicrobial tolerance throughout the challenging process. Phylogenomics and wgMLST analyses were carried out between the parent and resistant strain, as also the remaining isolates. Stable low and high-level resistant strains were successfully generated. Several genomic variants were identified in the high-level resistant strains. Validation of variant calling via PCR removed all miscalled variants. Comparative genome annotation revealed a high consistency in the genome structures of the clinical strains, despite non-consistent phylogenetic and synteny profiles. The mutation validation revealed several variations arising in genes responsible for signaling (*yihG bvgS* and *srrA*), metabolic activities (*atpD*, *ribonuclease I*, and *epsL*) and cell structure maintenance (*ftsI* and *yceG*) in addition to targeted mutations (*mexB*, *acrB* and *gyrA*). Analysis of the mutation chronology shows that when exposed to erythromycin, *A. baumannii* incurs modifications to genes *bvgS* and *srrA*, on days 4, 6, and to *ftsI* and its ribonuclease I encoding gene on day 67. When exposed to ciprofloxacin, mutations developed in *gyrA* and *yihG* on days 28 and 48. Meropenem exposure on the other hand has led to variations in *epsL*, *mexB*, and *atpD* on days 4, 10 and 70. In contrast, meropenem exposure resulted in mutations to *acrB* on day 38, and two mutations in *ftsI* occurred on day 19 and 67. From the results it is deduced that the chronology of intrinsic mutations is dependent on the types and intensity of selective pressures enacted, even on the same bacteria. Antibiotic pressure under *in vitro* and *in vivo* conditions has also resulted in development of different mutations leading to similar resistance profiles. It was also found that a prolonged exposure to the drugs used in this study plays as much of a role as the sub-inhibitory concentration.

ACKNOWLEDGEMENT

Graduate school has been one of the most exciting experiences I've had in discovering myself as a scientist. And I've had a lot of people to thank for putting up with my shenanigans throughout my studies.

Naturally, I thank my supervisors, Prof. Dr. Mohd Zaki Salleh and Prof. Dr. Teh Lay Kek for their unwavering dedication and patience in training me as their student. It goes without saying that without their aid, I would be nowhere close to where I am today.

My gratitude also goes to my family, especially my parents who have tolerated my unorthodox priorities during my studies. I am deeply gratified to my mother, Zaliha Bt. Ibrahim, who has been a source of comfort during my time of distress. And my father, Ismail Bin Mohd Salleh, to whom I am thankful for all the support he has given. I have long waited for the moment in which I can share this achievement with them. Although I feel the benchmark of success that they have set is far too high for me to reach, I hope I have done well enough to meet their expectations.

To my teachers and lecturers, which there are too many to name, I am eternally indebted to the knowledge they have imparted unto me. The passion for science that I have come to acquire is a result of the years I have spent learning from them. And although I admit I am a slow learner in certain aspects, they have never given up on me.

To my friends and colleagues, I am thankful for their assistance and company. Truth be told, my life as a postgraduate student would have been dull and colorless without them, especially, my headstrong bioinformatics team; Lee Lian Shien, Rose Ismet, and Shafiq Aazmi.

Finally, I am thankful to my best friend, Azira Azreen whom I have known since my first day as a university student. Our discourses and excursions were what kept me sane, and I have yet to find anyone of the same caliber as she.