UNIVERSITI TEKNOLOGI MARA

PHYLOGENETIC RELATIONSHIP OF NON-TYPEABLE *HAEMOPHILUS INFLUENZAE* ISOLATES

NURUL HAMIRAH KAMSANI

Thesis submitted in fulfilment of the requirements for the degree of Master of Science

Faculty of Medicine

December 2014
CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of examiners has met on 15th May 2014 to conduct the final examination of Nurul Hamirah Binti Kamsani on her Master of Science thesis entitled “Phylogenetic relationship of non-typeable Haemophilus influenza isolates” 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:

Zainal Abidin Abu Hasan, PhD
Professor
Faculty of Medicine
Universiti Teknologi MARA
(Chairman)

Noor Hana Hussain
Associate Professor
Faculty of Applied Science
Universiti Teknologi MARA
(Internal)

Zunita Zakaria
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(External)

SITI HALIJJA SHARIF, PhD
Associate Professor
Dean
Institute of Graduate Studies
Universiti Teknologi MARA
Date: 18th December, 2014
AUTHOR'S DECLARATION

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

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

Name of Student : Nurul Hamirah Binti Kamsani
Student I.D. No. : 2009234912
Programme : Master of Science (MD780)
Faculty : Medicine
Thesis : Phylogenetic Relationship of Non-typeable *Haemophilus influenzae* Isolates

Signature of Student : ...........................................
Date : December 2014
ABSTRACT

Nontypeable *Haemophilus influenzae* (NTHi) is a significant pathogen in children, causing otitis media, sinusitis, conjunctivitis, pneumonia, and occasionally invasive infections. Twenty-eight strains obtained from the Institute for Medical Research (IMR), Kuala Lumpur, were examined for antimicrobial susceptibility pattern and the relationship between the strains was determined by using three different molecular epidemiological methods; restriction fragment length polymorphism (RFLP), pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Antibiotic susceptibility test showed 33% of the strains were resistant to two or more of the tested antimicrobial agents. These NTHi strains demonstrated resistance most frequently to trimethoprim-sulfomethoxazole (32%), followed by ampicilin (21%), erythromycin (10%) and 7% each for chloramphenicol and streptomycin. Digestion with *Hin*I/III for RFLP demonstrated limited use because it produced only two to three bands for each strains. When analysed by PFGE, a total of 25 PFGE patterns were produced with *Sma*I, representing a genetically assorted population. It was observed that the strains fell into four major clusters with genetic distances >40% similarity of which only six strains were clonal with more than 90% similarity. While for MLST typing, new combination of allele numbers were found and 27 novel sequence types (STs) were detected. Among the three methods, RFLP was less discriminating in comparison to the PFGE and MLST. PFGE provides information on the relatedness between strains while the MLST reveals the genetic variation amongst Malaysian NTHi strains and their relationship to all the *H. influenzae* isolates in the MLST database. Based on all the three typing methods, this study shows that majority of the NTHi strains in Malaysia are heterogenous and are genetically diversified.
ACKNOWLEDGEMENTS

Alhamdulillah with Allah SWT consent, I finally completed my master degree. Though the report is in my voice, its text and illustrations are a synthesis of what I continue to learn from various references and experiments practiced. My name alone goes on the cover, but what is found between the covers is the result of many committed people working together toward the shared goal of improving science education. It is with a great deal of pressure that I thank those who have contributed in so many ways to the completion of the thesis.

First, with affectionate appreciation, I would like to thank my supervisor, Associate Professor Dr. Zaini Mohd Zain for her advice, cheerful spirit, guidance, encouragement for keeping me on track whenever I was about to slip by, gleaning errors from the text and problem sets that others missed. Without her supervision, it is almost impossible to get this thesis done.

Special thanks have to go to Associate Professor Sharifah Aminah as my co-supervisor from Faculty of Applied Science, UiTM Shah Alam for her careful guidance, contribution of ideas and many constructive and valuable suggestions.

I particularly want to thank Dr. Norazah from Institute of Research Medical (IMR), Kuala Lumpur and Professor Thong Kwai Lien from Faculty of Science, University of Malaya, I have learned so much from my work with them.

Most of all I am thankful beyond measure to my beloved parents, Puan Mashitah Binti Abdullah and Encik Kamsani Bin Saruni for their moral and financial support. To my family members, love ones and anyone who were involved in this project directly and indirectly, without whose patience and understanding, this thesis would never been written. May Allah SWT bless you.