UNIVERSITI TEKNOLOGI MARA

PHYLOGENETIC RELATIONSHIP OF NON-TYPEABLE *HAEMOPHILUS INFLUENZAE* ISOLATES

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

Faculty of Medicine

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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:

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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.

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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 *Hin1*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.

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