UNIVERSITI PUTRA MALAYSIA

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF STREPTOCOCCUS PNEUMONIAE ISOLATES AMONG HEALTHY CHILDREN IN KUALA LUMPUR AND SELANGOR, MALAYSIA

MASURA BINTI MOHD YATIM

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *STREPTOCOCCUS PNEUMONIAE* ISOLATES AMONG HEALTHY CHILDREN IN KUALA LUMPUR AND SELANGOR, MALAYSIA

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Chairman: Siti Norbaya binti Masri, MD, MPath

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There is scarce information about pneumococcal carriage among healthy children in Malaysia. The nasopharynx of human is well known as ecological reservoir of *Streptococcus pneumoniae* which is a precondition for developing pneumococcal diseases. Because the pneumococcal disease is common in children, this study was conducted to provide data on the prevalence rate, serotype distribution and antimicrobial susceptibility pattern of *S. pneumoniae* in the nasal carriage of healthy children. Further investigation such as pneumococcal surface protein A (PspA) family prevalence, clade distribution and its relatedness with pulsed-field gel electrophoresis (PFGE) patterns were also investigated.

Nasal swabs were collected from 195 healthy children age 5 years old or younger from June to December 2010 in three day care centers in Kuala Lumpur and Selangor. All *S. pneumoniae* isolates were successfully identified by both phenotypic and genotypic methods. The serotyping was performed using Pneumotest kit (Statens Serum Institut, Copenhagen, Denmark) and the antimicrobial susceptibility pattern was determined by using the E-test method (AB Biodisk, Solna, Sweden). PspA

family typing was done using polymerase chain reaction (PCR) and epidemiological study was investigated by PFGE.

S. pneumoniae was found in the nasal carriage of 35.4% (69/195) of children and this revealed an increasing trend of carriage prevalence among healthy children in Malaysia. Among the 69 isolates, penicillin resistant *S. pneumoniae* (PRSP) and multidrug resistant (more than two classes of antibiotic) *S. pneumoniae* (MDRSP) was 23.2% and 20.3% respectively. All 16 PRSP isolates were resistant to erythromycin and 14 PRSPs (87.5%) were resistant to cotrimoxazole. The six most common serotypes were 6A, 23F, 19A, 6B, 19F and 15C which were found in 87% of all isolates. The high rate of PRSP and MDRSP supports the need for continuing surveillance of pneumococcal carriage. In fact, data on surveillance of antimicrobial susceptibility pattern as well as serotype distribution also changed from time to time, emphasizing the need for continuing a surveillance study to keep in track the current situation.

Of the 69 isolates, 24.6% belonged to PspA Family 1, 71.0% were found to PspA Family 2 and 4.3% to PspA Family 3. With regard to vaccine serotypes coverage, 40.6% of the isolates belonged to serotypes included in the PCV7 and PCV10 and 81.1% included in the PCV13.

Even though conjugate vaccines from up to 13 serotypes have been developed, they only represent a limited number of serotypes, whereas over 90 serotypes exist. Due to the high cost and limited coverage, more effort is being focused to search for future vaccine candidates such as protein based vaccines which could cover the

whole population regardless of age as well as serotypes. By studying and exploring the pattern of distribution based on PspA typing, it would provide useful information for the suitability of this protein antigen as a vaccine candidate against pneumococcal population. In this finding, the major family was 1 and 2 (95.7%), thus making them suitable for future vaccines. In general, PFGE patterns of the pneumococcal isolates were genetically diverse, which suggest that the relationship between susceptibility pattern, PspA family types, PspA clades and PFGE patterns was independent.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

FENOTIPIK DAN PENCIRIAN GENOTIPIK ISOLAT *STREPTOCOCCUS PNEUMONIAE* DI KALANGAN KANAK-KANAK SIHAT DI KUALA LUMPUR DAN SELANGOR, MALAYSIA

Oleh

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Maklumat berkaitan pembawa pneumokokus di kalangan kanak-kanak sihat di Malaysia adalah terhad. Perkembangan penyakit pneumokokal bermula apabila saluran pernafasan manusia yang juga merupakan takungan ekologi semulajadi didiami oleh bakteria *Streptococcus pneumoniae*. Memandangkan penyakit pneumokokus biasanya menimpa kanak-kanak, kajian ini dijalankan bagi menyediakan data berkaitan prevalen, taburan serotaip dan corak kepekaan antimikrobial di kalangan kanak-kanak sihat di Malaysia. Kajian lanjutan seperti prevalen keluarga pneumococcal surface protein A (PspA), taburan clade dan hubungkaitnya dengan corak pulsed-field gel electrophoresis (PFGE) juga dikaji.

Sampel kesatan hidung diambil daripada 195 orang kanak-kanak sihat yang berumur lima tahun dan ke bawah mulai Jun sehingga Disember 2010 dari tiga buah pusat jagaan harian di Kuala Lumpur dan Selangor. Sebanyak 69 isolat *S. pneumoniae* berjaya dikenalpasti melalui kaedah fenotipik dan genotipik. Penjenisan serotaip ditentukan menggunakan kit pneumostest (Staten Serum Institut, Copenhagen, Denmark) dan corak kepekaan antimikrobial ditentukan menggunakan kaedah E-test

(AB Biodisk, Solna, Sweden). Pengkelasan keluarga PspA dijalankan menggunakan tindak balas rantai polimerase dan kajian epidemiologi dijalankan menggunakan PFGE.

Sebanyak 35.4% (69/195) *S. pneumoniae* dijumpai dari kesatan hidung kanak-kanak dan ini menunjukkan peningkatan berterusan kadar pembawa pneumokokus di kalangan kanak-kanak sihat di Malaysia. Daripada kesemua 69 isolat, kerintangan terhadap penicillin dan kebanyakan antibiotik (lebih daripada dua kelas antibiotik) adalah sebanyak 23.2% dan 20.3% masing-masing. Kesemua 16 isolat yang rintang terhadap penicillin didapati rintang terhadap erythromycin dan 14 isolat yang rintang terhadap penicillin juga rintang terhadap cotrimoxazole. Enam jenis serotaip yang biasa ditemui ialah 6A, 23F, 19A, 6B, 19F dan 15C dan ianya merangkumi sebanyak 87% daripada keseluruhan isolat. Kadar kerintangan yang tinggi terhadap penicillin dan kebanyakan antibiotik menunjukkan keperluan survelan berterusan perlu dilakukan terhadap pembawa pneumokokus. Malahan, data-data survelan seperti corak kepekaan antibiotik dan taburan serotaip yang sentiasa berubah-ubah dari masa kesemasa menekankan peri pentingnya survelan berterusan bagi mengenalpasti situasi semasa.

Daripada keseluruhan 69 isolat, 24.6% adalah terdiri daripada keluarga PspA jenis 1, 71.0% PspA jenis 2 dan 4.3% keluarga PspA jenis 3. Berkaitan dengan rangkuman serotaip vaksin, 40.6% daripada isolat adalah terangkum dalam serotaip vaksin konjugat pneumokokus 7 valen dan 10 valen masing-masing, manakala 81.1% terangkum dalam serotaip vaksin konjugat pneumokokus valen 13.

Meskipun vaksin konjugat pneumokokus sehingga 13 valen telah dibangunkan, ianya terhad mewakili sebahagian serotaip berbanding dengan lebih daripada dari 90 jenis serotaip yang sedia ada. Memandangkan kos yang tinggi dan liputan yang terhad, banyak usaha telah dijalankan bagi mencari vaksin-vaksin baru untuk kegunaan pada masa depan seperti vaksin berasaskan protin yang boleh meliputi keseluruhan populasi penduduk tanpa mengira peringkat umur dan serotaip. Dengan mengkaji dan mempelajari corak taburan berasaskan pengkelasan keluarga PspA, maka ia dapat memberi maklumat berguna berkaitan kesesuaian antigen protin sebagai calon vaksin terhadap populasi pneumokokus. Secara keseluruhan, kajian ini mendapati, sebahagian besar keluarga PspA adalah jenis 1 dan 2 iaitu sebanyak 95.7%, dengan itu sesuai sebagai calon vaksin pada masa hadapan.

Secara keseluruhannya, corak PFGE terhadap isolat pneumokokus menunjukkan corak genotaip yang pelbagai dan ini mencadangkan perhubungan di antara corak kepekaan antibiotik, pengkelasan keluarga PspA, PspA clade berbanding corak PFGE adalah tiada saling berhubungkait.

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I certified that a Thesis Examination Committee has met on 18 September 2013 to conduct the final examination of Masura binti Mohd Yatim on her thesis entitled "Phenotypic and Genotypic Characterization of *Streptococcus pneumoniae* Isolates among Healthy Children in Kuala Lumpur and Selangor, Malaysia" in accordance with the Universities and Universities Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory were as follows:

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Date: 12 December 2013

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

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MASURA BINTI MOHD YATIM

Date: 18 September 2013

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