



UNIVERSITI PUTRA MALAYSIA

**DISTRIBUTION OF SEROGROUPS AND GENOTYPES AMONG
NEISSERIA MENINGITIDIS STRAINS IN MALAYSIA**

PUVANESVARI KUPPUSAMY

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STRAINS IN MALAYSIA**

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**MASTER OF SCIENCE
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By

PUVANESVARI KUPPUSAMY

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

June 2009



DEDICATION

I dedicate this piece of work to my beloved parents. Thank you for all your love, encouragements and support. Love you with all my heart.



Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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NEISSERIA MENINGITIDIS STRAINS IN MALAYSIA**

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PUVANESVARI KUPPUSAMY

June 2009

Chairman: Professor Datin Dr. Farida Fatema @ Farida Jamal

Faculty: Faculty of Medicine and Health Sciences

Neisseria meningitidis causes meningococcal disease, a life threatening illness with an annual incidence of 1 to 1000 per 100,000 population in different parts of the world and humans are the only known host to this organism. The fastidious nature of the organism and the common practice of starting antibiotic treatment prior to sample collection pose a challenge in culture based diagnosis of this organism. Typing methods based on molecular techniques are also replacing the phenotypic methods such as serogrouping owing to frequent lack of expression of the surface antigens as well as the unavailability of the typing antisera. Increased reports of *N. meningitidis* showing decrease susceptibility to penicillin, the drug of choice in meningococcal disease treatment has also raised some concern.



In this study, a novel multiplex PCR assay was developed to identify *N. meningitidis*, detect its capsule (an important virulence factor in bloodstream invasion) and determine its susceptibility to penicillin. In addition, a multiplex PCR assay to determine the serogroups A, B, C, Y and W135 as well as a duplex PCR assay to determine the serogroups X and Z were adapted and optimized from previously published work. These assays were used against 123 *N. meningitidis* strains (114 carrier strains and 9 clinical strains). All the strains had first been identified by biochemical reactions, serogrouped by the slide agglutination technique and tested for susceptibility to penicillin by the Etest® method. The gene targeted for identification (*porA*) was detected in all the 123 test strains and all 7 reference meningococci strains but not in other *Neisseria* species, making it a good marker for identification. The capsule gene (*ctrA* gene) was detected in 8/9 (88.9%) clinical strains and 35/114 (30.7%) carrier strains. The lack of capsule among the carrier strains, is high but non-capsulated strains among carriers is a common phenomenon. The susceptibility to penicillin gene (*penA*) was detected in 8/9 (88.9%) clinical strains and 74/114 (64.9%) carrier strains, thus 33.3% of all strains showed intermediate resistance to penicillin. Detection of serogroups by PCR (genogrouping) showed an increase of 86% when compared to the slide agglutination technique, and this increase was seen only among the carrier strains. 21.2% of the strains were genogroupable showing predominance of serogroups B and W135 in clinical strains and Y, B, Z, W135 and X among carrier strains (in decreasing order).



Multilocus Sequence Typing (MLST) was used to genotype all the clinical strains and 71 of the carrier strains. A total of 32 sequence types (STs) of which 21 novel STs unique to Malaysia were found in this study showing a population of high genetic diversity. Of the 21 novel STs, 17 were unique for the carrier strains while 3 were unique for the clinical strains. Only one novel ST, ST7129 was found in both the carrier and clinical strains. Six clonal complexes of which two were of hyper invasive lineage, ST-11 Complex/ET-37 Complex (2.6%) and ST-41/44 Complex/ Lineage 3 (20.2%) were detected among the strains. Burst analysis identified three ancestral genotypes among the studied strains. These results indicate potential application of MLST to the study of meningococcal epidemiology and evolution in Malaysia. While the genogrouping results show the potential of using these multiplex PCR assays in detecting the serogroups of the *N. meningitidis* strains, especially strains designated as “non- serogroupable” by the conventional methods.

*Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Sarjana Sains*

**TABURAN KUMPULAN SERA DAN GENOTAIP DI KALANGAN STRAIN
NEISSERIA MENINGITIDIS DI MALAYSIA**

Oleh

PUVANESVARI KUPPUSAMY

Jun 2009

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Neisseria meningitidis menyebabkan penyakit meningokokal, yang merupakan penyakit merbahaya dengan kadar insiden tahunan 1 bagi setiap 1000 penduduk di pelbagai pelusuk dunia dan diketahui hanya manusia sahaja yang menjadi perumah untuk organisma ini. Keadaan semulajadi yang cerewet bagi organism ini dan amalan mengambil rawatan antibiotik sebelum pengambilan sampel merupakan cabaran dalam pendiagnosaan organisma yang berasaskan kultur ini. Kaedah pentaipan berasaskan teknik molekular juga menggantikan kaedah fenotaip seperti serotaip organism yang gagal mengekspresikan antigen permukaan disamping ketiadaan antisera untuk pentaipannya. Peningkatan laporan tentang *N. meningitidis* menunjukkan penurunan kepekaan terhadap antibiotik penisilin yang merupakan pilihan untuk rawatan penyakit meningokokal juga telah menarik perhatian.



Dalam kajian ini, kaedah penganalisan PCR multipleks telah ditemui untuk mengenalpasti *N. meningitidis*, mengesan kapsul (faktor virulen yang penting di dalam pengaliran darah) dan pengesanan gen untuk kepekaan kepada penisilin. Kajian ini telah menggunakan 123 strain *N. meningitidis* (114 strain pembawa dan 9 strain klinikal). Semua strain telah dicerap menggunakan ujian reaksi biokimia, kumpulan sera secara teknik aglutinasi slaid dan diuji untuk kepekaan terhadap penisilin menggunakan kaedah Etest®. Gen sasaran untuk identifikasi (*porA*) yang telah dikesan di dalam kesemua 123 strain ujian dan semua 7 strain rujukan tetapi tidak dalam spesis *Neisseria* yang lain, merupakan petunjuk yang baik untuk identifikasi. Gen kapsul (gen *ctrA*) telah dikesan di dalam 8/9 (89.9%) strain klinikal dan 35/114 (30.7%) strain pembawa. Ketiadaan kapsul pada strain pembawa adalah tinggi, tetapi strain tanpa kapsul diantara pembawa adalah fenomena yang biasa. Kepekaan terhadap gen penisilin (*penA*) telah dikesan dalam 8/9 (89.9%) strain klinikal dan 74/114 (64.9%) strain pembawa, yakni 33.3% daripada kesemua strain menunjukkan kerintangan sederhana terhadap penisilin. Pengesanan kumpulan sera menggunakan PCR (kumpulan geno) menunjukkan peningkatan sebanyak 86% berbanding dengan teknik aglutinasi slaid. 21.2% daripada strain telah dapat dibuat kumpulan geno dimana kebanyakannya kumpulan sera B dan W135 di dalam strain klinikal dan Y, B, Z, W135 dan X di kalangan strain pembawa (dalam urutan menurun).

Pentaipan Jujukan Pelbagai Lokus (MLST) telah digunakan untuk pentaipan gen kesemua strain klinial dan 71 daripada strain pembawa. Sejumlah 32 pentaipan jujukan (STs) ditemui, di mana 21 ST adalah baru dan unik kepada Malaysia. Ini menunjukkan populasi *N. meningitidis* yang telah dikaji mempunyai kepelbagaian genetic yang tinggi. Daripada penemuan 21 STs tersebut, 17 adalah unik untuk strain pembawa sementara 3 adalah unik untuk strain klinikal. Satu sahaja penemuan ST, ST7129 telah ditemui dalam kedua-dua strain pembawa dan klinikal. Enam kompleks ditemui dikalangan strain yang dikaji, di mana dua kompleks, ST-11 kompleks/ET-37 kompleks (2.6%) dan ST-41/44 kompleks (20.2%), adalah tinggi barisan penerapannya. Hasil dari analisa ini, 3 genotaip telah dikenalpasti sebagai strain pemula kompleks yang ditemui. Keputusan ini menunjukkan potensi aplikasi MLST untuk kajian evolusi dan epidemiologi meningokokal di Malaysia. Keputusan pengesanan kumpulan sera melalui PCR juga mempunyai potensi sebagai alat pendiagnosaan *N. Meningitidis*, terutama strain yang tidak dapat di kesan kumpulan seranya menggunakan kaedah biasa.

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I certify that an Examination Committee has met on 30th June 2009 to conduct the final examination of Puvanesvari Kuppusamy on her Master of Science thesis entitled “Distribution of Serogroups and Genotypes Among *Neisseria meningitidis* Strains In Malaysia” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the (Name of relevant degree).

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DECLARATION

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

PUVANESVARI KUPPUSAMY

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LIST OF ABBREVIATIONS

°C	Degree celcius
µg	Microgram
µl	Microliter
µM	Micromolar
<i>abcZ</i>	Genes encoding putative ABC transporter
<i>adk</i>	Genes encoding adenylate kinase
<i>aroE</i>	Genes encoding shikimate dehydrogenase
ATCC	American Type Culture Collection
BA	Blood agar
bp	Base pair
CA	Chocolate agar
CLSI	The Clinical and Laboratory Standards Institute
CMP	Cytidine monophospate
<i>cps</i>	Capsule locus
<i>crgA</i>	Gene encoding a transcriptional regulator belonging to the Lysine R family
CSF	Cerebrospinal fluid
<i>ctr</i>	Gene encoding an outer membrane protein involved in capsule transport
DNA	Deoxyribonucleic acid



ET	Enzyme type
FPSK	Faculty of Medicine and Health Sciences
FRU	Fructose
<i>fumC</i>	Genes encoding fumarate hydratase
<i>gdh</i>	Genes encoding glucose-6-phosphate dehydrogenase
GGT	Gamma glutamyl tranferase
GLU	Glucose
I	Intermediate
IMR	Institute of Medical Research
IND	Indole
IS	Insertion sequence
LIP	Lipase
LOS	Lipooligosaccharide
MAL	Maltose
MIC	Minimum inhibitory concentration
min	Minutes
ml	Milliliter
MLEE	Multilocus enzyme electrophoresis
MLST	Multilocus sequence typing
MLVA	Multilocus variable number tandem
MT	Gene encoding methyltransferase
<i>myn</i>	Gene encoding polymannosetransferase

NANA	N-acetylneuraminic acid
NC	Negative control
NCCLS	National Committee for Clinical Laboratory Standard
NPHL	National Public Health Laboratory
<i>nspA</i>	Gene encoding <i>Neisseria</i> surface protein A
ODC	Ornithine decarboxylase
OMP	Outer membrane proteins
orf	Open reading frame
PAL	Alkaline phosphatase
PBP	Penicillin-binding protein
PC	Positive control
PCR	Polymerase Chain Reaction
<i>pdhC</i>	Genes encoding pyruvate dehydrogenase subunit
<i>penA</i>	Gene encoding PBP2
PFGE	Pulse field gel electrophoresis
<i>pgm</i>	Genes encoding phosphoglucomutase
Por	Porin
ProA	Proline arylamidase
psi	Pound per square inch
R	Resistant
RAPD	Random amplification of polymorphic DNA
RC	Reagent control

