UNIVERSITI PUTRA MALAYSIA

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

PUVANESVARI KUPPUSAMY

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**MASTER OF SCIENCE**
**UNIVERSITI PUTRA MALAYSIA**

**2009**

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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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**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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**Faculty: Fakulti Perubatan dan Sains Kesihatan**

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 penganalisaan PCR multipleks telah ditemui untuk mengenalpasti *N. meningitidis*, mengesan kapsul (faktor virulen yang penting di dalam pengaliran darah) dan pengesana 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 pensilin. 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 kebanyakkannya kumpulan sera B dan W135 di dalam strain klinikan 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 menunjukan 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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All my friends and family, words cannot express my gratitude. Thank you for all your support and words of encouragement. You all were my ray of sunshine on cloudy days.

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 awarde the (Name of relevant degree).

Members of the Examination Committee were as follows:

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

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Date: 17 July 2009

## 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.

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**PUVANESVARI KUPPUSAMY**

Date:

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| | |
|---|---|
| °C | Degree celcius |
| µg | Microgram |
| µl | Microliter |
| µM | Micromolar |
| *abcZ* | Genes encoding putative ABC transporter |
| *adk* | Genes encoding adenylate kinase |
| *aroE* | 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 |
| *cps* | Capsule locus |
| *crgA* | Gene encoding a transcriptional regulator belonging to the Lysine R family |
| CSF | Cerebrospinal fluid |
| *ctr* | 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 |
| *fumC* | Genes encoding fumarate hydratase |
| *gdh* | 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 methyltranferase |
| *myn* | Gene encoding polymannosetransferase |

| | |
|---|---|
| NANA | N-acetylneuraminic acid |
| NC | Negative control |
| NCCLS | National Committee for Clinical Laboratory Standard |
| NPHL | National Public Health Laboratory |
| *nspA* | Gene encoding *Neisseria* 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 |
| *pdhC* | Genes encoding pyruvate dehydrogenase subunit |
| *penA* | Gene encoding PBP2 |
| PFGE | Pulse field gel electrophoresis |
| *pgm* | Genes encoding phosphoglucomutase |
| Por | Porin |
| ProA | Proline arylamidase |
| psi | Pound per square inch |
| R | Resistant |
| RAPD | Random amplification of polymorphic DNA |
| RC | Reagent control |