



**UNIVERSITI PUTRA MALAYSIA**

***DISTRIBUTION OF SEROTYPES AND VIRULENCE GENES AMONG  
INVASIVE, NON-INVASIVE AND COLONIZING *Streptococcus  
agalactiae* (GROUP B STREPTOCOCCUS) ISOLATES FROM  
PATIENTS IN A MAJOR TEACHING HOSPITAL IN MALAYSIA***

**NARGES ESKANDARIAN**

**FPSK(m) 2014 2**



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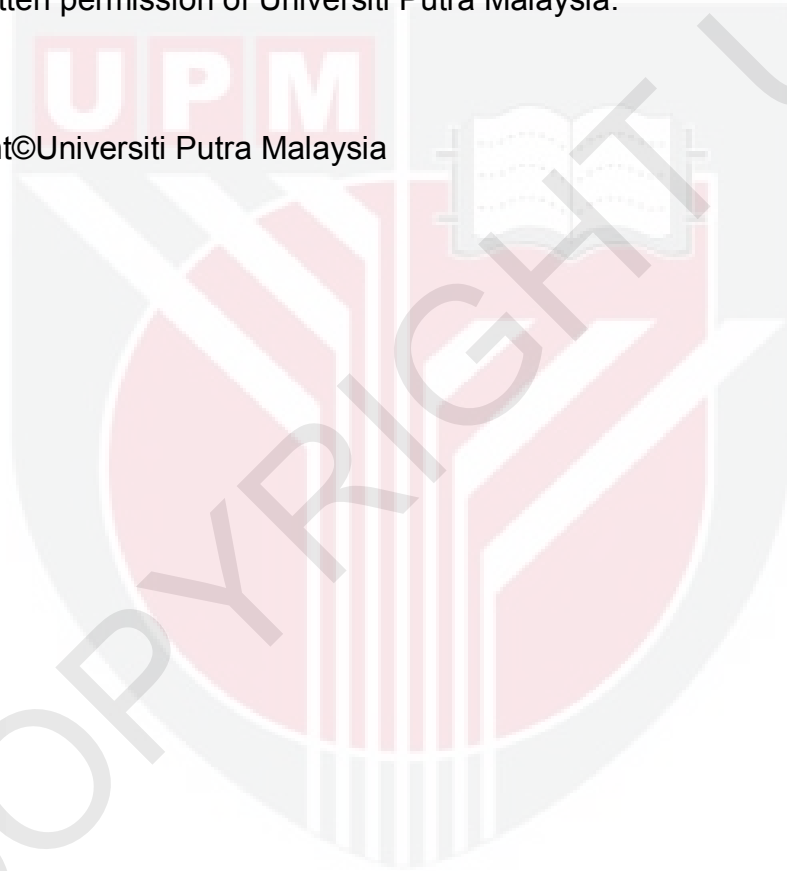
**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfillment of the Requirements for the Degree of Master of  
Science**

**January 2014**

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## DEDICATION

I dedicate this piece of work to my parents and my beloved husband  
Vahid. Thank you all for all your love, encouragement and support.

Love you with all my heart



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements of the degree of Master Science

**DISTRIBUTION OF SEROTYPES AND VIRULENCE GENES AMONG  
INVASIVE, NON-INVASIVE AND COLONIZING *Streptococcus agalactiae*  
(GROUP B STREPTOCOCCUS) ISOLATES FROM PATIENTS IN A  
MAJOR TEACHING HOSPITAL IN MALAYSIA**

By

**NARGES ESKANDARIAN**

**January 2014**

**Chairman: Syafinaz Amin Nordin, MBChB, MPath (Med. Microbiology)**

**Faculty: Medicine and Health Sciences**

*Streptococcus agalactiae* also known as group B streptococcus (GBS) remains a significant cause of neonatal sepsis and meningitis worldwide. Approximately 30% of healthy women are colonized with GBS. Main reservoirs are the gastrointestinal and genitourinary tract. GBS pose major threat to the women during their prenatal stage, which are transmitted to fetus during delivery resulting in serious infections and occasional deaths in neonates. It causes early onset diseases (EOD) within 7 days of birth and late onset disease (LOD) from 7 to 90 days. Apart from pregnant women and neonates, GBS infections are also seen in immunocompromised, and elderly patients. Numerous prevention strategies for the control of GBS-related neonatal infections including antibiotic prophylaxis and vaccination have been practiced in many parts of the world. GBS strains vary from country to country; hence, there is a variation in serotype distribution, antibiotic susceptibility and virulence factors of GBS strains, which challenge the management of GBS related infections. In Malaysia, despite of several cases of GBS-related neonates' infections, data concerning the serotype epidemiology, antibiotic susceptibility and the virulence pattern of local strains are lacking. Therefore, the current study was aimed at determining the serotypes, antibiogram and the virulent gene profile of Malaysian GBS strains. Hundred and three pure cultures of *S. agalactiae* were obtained from Universiti Kebangsaan Malaysia Medical Center (UKMMC) from June 2010 to October 2011, included 22 invasive, 23 non-invasive and 58 colonizing

strains were isolated from clinical samples. Capsular serotyping was performed with the latex agglutination method using specific antisera against types Ia, II-VII CPS antigens. The results of the conventional serotyping (CS) of the strains were further confirmed by molecular serotyping (MS). All conventionally identified serotypes were in agreement with the molecular serotype. Twenty (19.4%) isolates that were non-typable by CS, were also typed by MS. Serotype VI (22.3%), VII (21.3%) were the most common serotypes, and serotype IV (1%) was found to be the least. Antimicrobial susceptibility testing carried out to determine the susceptibility of GBS isolates revealed that all isolates were susceptible to penicillin by both disk diffusion and E-test methods. No resistance was observed for cefuroxime, ceftriaxone, levofloxacin and vancomycin, while resistance rate of 23.3%, 16.5% and 71.8% were seen for erythromycin, clindamycin and tetracycline respectively. Seven virulence genes (*cylE*, *lmb*, *scpB*, *hylB*, *rib*, *bca*, *bac*) screened on the studied isolates showed the presence of *cylE*, *lmb*, *scpB* and *hylB* in almost all the isolates while *rib*, *bca*, *bac* genes were found to be present in 29.1%, 14.6% and 9.7% of the isolates. The present study for the first time reports the detection of virulence genes in Malaysian strains. A significant association between *rib* gene and serotypes Ia, II, III and VI; *bca* with serotypes II, III and VI; *bac* with serotypes II and III were observed. In conclusion, this study demonstrated that VI and VII as the predominant serotypes in Malaysia, hence need to be considered while developing vaccines. The antibiotic susceptibility pattern showed local strains are susceptible to penicillin and demonstrated it still could be used as the first choice for GBS treatment. Significant association of virulence factors with certain serotypes need to be routinely monitored to reduce the GBS associated mortality.

Abstrak tesis yang telah dikemukakan kepada Senat Universiti Putra  
Malaysia sebagai memenuhi keperluan ijazah Master Sains

**TABURAN SEROTIP DAN GEN VIRULEN PADA ISOLAT *Streptococcus agalactiae* (STREPTOCOCCUS KUMPULAN B) INVASIF, TIDAK INVASIF DAN JAJAHAN DARIPADA PESAKIT HOSPITAL PENGAJARAN UTAMA DI MALAYSIA**

Oleh

**NARGES ESKANDARIAN**

**Januari 2014**

**Pengurus: Syafinaz Amin Nordin, MBChB, MPath (Med. Microbiology)**

**Fakulti: Perubatan dan Sains Kesihatan**

*Streptococcus agalactiae* juga dikenali sebagai streptococcus kumpulan B (GBS), kekal sebagai penyebab utama sepsis neonat dan meningitis di seluruh dunia. Dianggarkan 30% wanita sihat adalah pembawa GBS. Organisma ini biasanya ditemui di dalam gastrousus dan saluran genitourinari. Wanita hamil yang membawa GBS berisiko untuk menjangkitkan organisma ini kepada fetus sewaktu proses kelahiran dan mengakibatkan jangkitan yang serius pada neonat serta kematian dalam sesetengah kes. GBS menyebabkan early onset disease (EOD) dalam tempoh tujuh hari kelahiran dan late onset disease (LOD) dari hari ketujuh hingga ke sembilan puluh kelahiran. Selain daripada wanita hamil dan neonat, jangkitan GBS juga dilihat di kalangan pesakit yang mengalami ketidakupayaan sistem imun mereka serta mereka yang berusia. Pelbagai strategi pencegahan untuk membasmi jangkitan GBS di kalangan neonat telah diamalkan di seluruh dunia, termasuk penggunaan antibiotik (prophylaxis) dan vaksin. Pelbagai faktor memberi cabaran untuk merawat jangkitan GBS, iaitu kepelbagaian serotip GBS, corak kerentanan antibiotik serta gen virulen yang berbeza dari satu negara ke negara yang lain. Di Malaysia, walaupun terdapat kes-kes jangkitan GBS di kalangan neonat, informasi tentang epidemiologi serotip, corak kerentanan antibiotik dan gen virulen tidak banyak didapati. Oleh itu kajian ini bertujuan untuk menentukan taburan serotip, corak kerentanan antibiotik dan profil gen virulen di kalangan isolat GBS di Malaysia. Seratus tiga isolat GBS telah dikumpulkan dari sampel klinikal di Universiti Kebangsaan Malaysia dari Jun 2010 sehingga

Oktober 2011, yang terdiri daripada 22 isolat invasif, 23 isolat tidak invasif dan 58 isolat colonizing. Penentuan serotip kapsular dilakukan dengan kaedah gumpalan lateks yang menggunakan antisera khusus terhadap antigen jenis Ia, CPS II-VII. Pengesanan jenis kapsular dilakukan secara kaedah molekul (PCR) bagi semua jenis strain termasuk isolat yang telah tidak dapat ditentukan melalui kaedah lateks. Keputusan penentuan serotip yang dilakukan secara konvensional lateks (CS) dan molekular (MS) menunjukkan persamaan. Dua puluh (19.4%) isolat yang tidak dapat ditentukan secara CS telah dapat ditentukan melalui kaedah MS. Serotip VI (22.3%) dan VII (21.3%) adalah serotip yang paling banyak dan serotip IV (1%) yang paling kurang dijumpai. Ujian kerentanan antibiotik menunjukkan semua isolat adalah rentan kepada penisilin melalui kaedah penyerapan disk dan E-test. Tiada bakteria yang rintang terhadap cefuroxime, ceftriaxone, levofloxacin dan vancomycin dijumpai, sementara bakteria rintang terhadap erythromycin, clindamycin dan tetracycline adalah masing-masing sebanyak 23.3%, 16.5% dan 71.8%. Pengesanan tujuh gen virulen (*cylE*, *lmb*, *scpB*, *hylB*, *rib*, *bca*, *bac*) melalui kaedah PCR menunjukkan kehadiran gen *cylE*, *lmb*, *scpB* dan *hylB* pada semua isolat sementara gene *rib*, *bca* dan *bac* masing-masing dikesan pada 29.1%, 14.6% dan 9.7% isolat. Kajian ini adalah yang pertama melaporkan pengesanan virulen gen di dalam isolat GBS di Malaysia. Perkaitan yang signifikan telah ditemui antara gen *rib* dan serotip Ia, II, III dan VI; gen *bca* dan serotip II dan III. Secara kesimpulan, kajian ini menunjukkan serotip VI dan VII adalah serotip paling dominan di Malaysia dan boleh digunakan dalam penghasilan vaksin GBS. Ujian kerentanan antibiotik menunjukkan GBS di Malaysia masih rentan kepada penisilin dan boleh digunakan sebagai kaedah rawatan pertama. Hubungan yang signifikan di antara faktor virulen dan serotip tertentu perlu dipantau secara rutin untuk mengurangkan kes jangkitan GBS yang menyebabkan kematian.



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Thanks also to the authority of Universiti Putra Malaysia for providing fund (04-04-10-1002RU/F1) to conduct the present research.

I certify that a Thesis Examination Committee has met on 7 January 2014 to conduct the final examination of Narges Eskandarian on her thesis entitled "Distribution of Serotypes and Virulence Genes among Invasive, Non-Invasive, and Colonizing *Streptococcus agalactiae* (Group B Streptococcus) Isolates from Patients in A Major Teaching Hospital in Malaysia" in accordance with the Universities and University 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.

Members of the Thesis Examination Committee were as follows:

**Malina binti Osman, PhD**

Senior Lecturer  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Chairman)

**Zamberi bin Sekawi, PhD**

Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Internal Examiner)

**Latifah binti Saiful Yazan, PhD**

Associate Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Internal Examiner)

**Parasakthi Navaratnam, PhD**

Senior Lecturer  
Monash University Sunway Campus  
Malaysia  
(External Examiner)



---

**NORITAH OMAR, PhD**

Associate Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 10 March 2014

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 Committee were as follows:

**Syafinaz Amin Nordin, MBChB, MPath**

Senior Lecturer  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Vasantha Kumari Neela, PhD**

Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

**Zalina Ismail, MBBCh, BAO, MPath**

Senior Medical Lecturer  
Faculty of Medicine  
Universiti Kebangsaan Malaysia  
(Member)

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**BUJANG BIN KIM HUAT, PhD**

Professor and Dean  
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## DECLARATION

### Declaration by graduate student

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Signature: \_\_\_\_\_  
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Member of  
Supervisory  
Committee: \_\_\_\_\_

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