



**UNIVERSITI PUTRA MALAYSIA**

***ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AND MOLECULAR  
CHARACTERISTICS OF GROUP B STREPTOCOCCUS ISOLATED FROM  
THREE MAJOR HOSPITALS IN MALAYSIA***

**MOHD. EMIR SHAFIQ BIN SUHAIMI**

**FPSK(m) 2015 40**



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**By**

**MOHD. EMIR SHAFIQ BIN SUHAIMI**

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

**February 2015**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

**ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AND MOLECULAR CHARACTERISTICS OF GROUP B *STREPTOCOCCUS* ISOLATED FROM THREE MAJOR HOSPITALS IN MALAYSIA**

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**February 2015**

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Group B *Streptococcus* (GBS) or also known as *Streptococcus agalactiae* can cause sepsis and meningitis in neonates and it also can promote serious mortality in immunocompromised patients and morbidity in pregnant women and non-pregnant adults. The Centers for Disease Control and Prevention (CDC) published guidelines for intrapartum antibiotic prophylaxis since 1996 and recommended penicillin or ampicillin as the drugs of choice for prevention of GBS disease. The objective of this study is to analyze distribution of serotypes and antimicrobial susceptibility patterns of sixty GBS isolates and their potential genetic relation by Random Amplified of Polymorphic DNA (RAPD) analysis so that it acts as an additional reference for intrapartum chemoprophylaxis against GBS infection. All GBS isolates were obtained from different patients admitting to Hospital Serdang, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) and Hospital Kuala Lumpur from December 2012 until March 2014. Ten isolates from each invasive and colonizing site were collected from each hospital. Disc diffusion test used as pre-screening for detection of antimicrobial susceptibility of GBS isolates. D test was applied to check any inducible resistant for clindamycin after being induced by erythromycin and E-test was used to detect minimal inhibitory concentration (MIC) for GBS isolates resistant or intermediate to erythromycin, clindamycin and penicillin. Serotyping was performed by latex agglutination method using specific antisera against type Ia, Ib, II until IX antigens. The process was confirmed further by molecular approach using multiplex PCR. The genetic relation between the isolates was examined by RAPD typing using P2 and GBS2 primer. Composite dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method. Ninety-eight percent of the isolates (n = 59) were susceptible with penicillin (one isolate was recorded intermediate sensitive to penicillin) whereas 78 % (n = 47) and 88 % (n = 53) were susceptible to erythromycin and clindamycin. Serotype Ia was most common serotype, (n = 27, 45 %) followed by serotype III (n=10, 16.7%), V (n = 9, 15%), VI (n = 8, 13.3%), II (n = 3; 5 %) VIII and VII (n = 2, n = 1; 3.3%, 1.7%). None for serotype Ib, IV and IX.

Based on RAPD, the study showed a diverse genetic pedigree of all isolates in four major clusters. At 31 % of similarity, isolates were segregated in the dendrogram according to their isolation sites and location of hospitals. There were no obvious genetic linkages in relation to antimicrobial patterns and serotypes. This study preliminarily suggest the occurrence of distinct GBS isolates in RAPD dendrogram and most of the GBS isolates that resistant toward erythromycin and clindamycin come from serotype III although serotype Ia being the most common detected in GBS isolates regardless of the isolation site and location.



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**CORAK KERENTANAN ANTIMIKROBIAL DAN CIRI-CIRI MOLEKULAR  
KUMPULAN B STREPTOCOCCUS DIASINGKAN DARI TIGA HOSPITAL  
UTAMA DI MALAYSIA**

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Kumpulan B Streptococcus (GBS) atau lebih dikenali sebagai *Streptococcus agalactiae* boleh menyebabkan sepsis dan meningitis terhadap bayi dan ia juga boleh membawa kepada kematian yang serius kepada pesakit-pesakit yang terimunokompromi dan morbiditi kepada wanita hamil dan dewasa. Pusat Kawalan Penyakit dan Pencegahan (CDC) telah menggariskan panduan untuk intrapartum antibiotik prophylaxis sejak tahun 1996 dan telah mengesyorkan penicillin dan ampicillin sebagai antibiotik pilihan untuk mencegah penyakit GBS. Objektif penyelidikan ini adalah untuk menganalisa pengagihan serotip dan corak kerentanan antimikrobial terhadap enam puluh isolat GBS dan potensi perhubungan genetiknya melalui kaedah Amplifikasi Polimorf DNA Rawak (RAPD) agar ianya boleh dijadikan sebagai rujukan tambahan untuk intrapartum chemprophylaxis terhadap jangkitan GBS. Semua isolat GBS diperolehi daripada pesakit yang berbeza dan telah dimasukkan ke Hospital Serdang, Pusat Perubatan Universiti Kebangsaan Malaysia (PPUKM) dan Hospital Kuala Lumpur dari Disember 2012 hingga Mac 2014. Sepuluh isolat daripada sumber invasif dan jajahan telah dikumpul dari setiap hospital. Ujian resapan cakera telah digunakan sebagai pra-ujian untuk mengesan kerentanan antimikrobial terhadap isolat GBS. Ujian D telah digunakan untuk mengesan rintangan terhadap clindamycin selepas didorong daripada erythromycin dan E-test telah digunakan untuk mengesan kepekatan rintangan yang minima (MIC) untuk semua isolat GBS yang mempunyai rintangan dan separa rintangan terhadap erythromycin, clindamycin dan penicillin. Ujian serotip telah dijalankan melalui kaedah lateks aglutinasi dengan menggunakan antisera yang spesifik terhadap jenis antigen Ia, Ib, II hingga IX. Proses ini telah dipastikan melalui kaedah molekular dengan menggunakan kaedah PCR multipleks. Hubungan genetik antara isolat telah diuji melalui kaedah RAPD dengan menggunakan primer P2 dan GBS2. Rajah pokok komposit telah dibina dengan menggunakan kaedah Kumpulan Pasangan Unweighted dengan Kaedah Aritmetik Min (UPGMA). Sembilan puluh lapan peratus isolat (n = 59) telah rentan terhadap penicillin (satu isolat telah direkod separa rentan

terhadap penicillin) manakala 78 % (n = 47) dan 88 % (n = 53) telah rentan terhadap erythromycin dan clindamycin. Serotip Ia adalah serotip yang paling banyak dikenalpasti (n = 27, 45 %) diikuti dengan serotip III (n = 10, 16.7%), V (n = 9, 15%), VI (n = 8, 13.3%), II (n = 3; 5 %) VIII dan VII (n = 2, n = 1; 3.3%, 1.7%). Tiada serotip yang dikenalpasti untuk serotip Ib, IV and IX. Berdasarkan kaedah RAPD, hasil penyelidikan telah menunjukkan salasilah genetik yang luas dan telah membahagikan semua isolat kepada empat kluster major. Pada kadar 31 % kesamaan, semua isolat di dalam dendrogram telah dibahagikan mengikut sumber isolat dan lokasi hospital. Tiada hubungan kesamaan genetik yang jelas terhadap corak antimikrobial dan serotip. Penyelidikan ini mencadangkan kehadiran hubungan genetik yang jauh antara semua isolat GBS di dalam rajah pokok RAPD dan kebanyakan isolat GBS yang rintang terhadap erythromycin dan clindamycin datang daripada serotip III walaupun serotip Ia merupakan serotip yang paling banyak dikesan ke atas semua isolat GBS tanpa mengira sumber isolat dan lokasi.

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I certify that a Thesis Examination Committee has met on 25 February 2015 to conduct the final examination of Mohd. Emir Shafiq Bin Suhaimi on his thesis entitled “Antimicrobial Susceptibility Patterns and Molecular Characteristics of Group B *Streptococcus* Isolated from Three Major Hospitals 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.

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## LIST OF ABBREVIATIONS

ATCC	- American type Culture Collection
BLAST	- The Basic Local Alignment Search Tool
CPS	- Capsular polysaccharides
CS	- Capsular serotyping
HKL	- Hospital Kuala Lumpur
MHA	- Mueller Hinton Agar
MS	- Molecular serotyping
PCR	- Polymerase Chain Reaction
UKMMC	- UniversitiKebangsaan Malaysia Medical Centre
RAPD	- Random Amplified of Polymorphic DNA
rRNA	- ribosomal RNA
<i>Strep.</i>	- <i>Streptococcus</i>
TBE	- Tris-borate-EDTA
UPGMA	- Unweighted Pair-Group Method Arithmetic Averaged
UPM	- Universiti Putra Malaysia

## CHAPTER I

### INTRODUCTION

Group B Streptococcus (GBS) or also known as *Streptococcus agalactiae* is Gram-positive bacteria in bacterial cocci group, produced a positive CAMP test and negative result in catalase test. GBS are usually located at pregnant woman's vagina and normally colonize in 50 % of healthy adult's gastrointestinal and genitourinary tract (Schuchat, 1998). In terms of pathogenicity, GBS can cause pneumonia, sepsis and meningitis in neonates which they are infected via vertical transmission from their mother (Turner *et al.*, 2002). GBS also has been recognized to cause serious mortality in immunocompromised patients and significant morbidity in pregnant woman and elderly since 1970s (Berardi *et al.*, 2007; Edwards & Baker, 2005; Schuchat, 1999).

GBS infection's period in neonates can be listed either early onset disease (EOD) which occur on the first day of baby's delivery until six days of life and late onset disease (LOD) which occur on 7<sup>th</sup> day of age until 90 days (Fluegge *et al.*, 2006). Recently, GBS was recognized as an important cause of invasive disease in non-pregnant adults and diabetes mellitus is the most common disease in elderly (Farley, 2001). Invasive GBS cases continue to rise although there is recent success of prevention efforts on neonatal GBS infection (Farley, 2001). It is a serious problem worldwide and steps need to be taken to reduce the case.

Capsular polysaccharide (*cps*) is assumed as a virulence factors as well as targets for protective immunity (Watanabe *et al.*, 2002). Recent research until now concluded that there are generally 10 *cps* of GBS serotypes which are Ia, Ib, II, III, IV, V, VI, VII, VIII and the latest serotype, IX which was identified in 2010 (Imperi *et al.*, 2010; Slotved *et al.*, 2007). Generally, serotype Ia, III and V are commonly present in the pregnant women and neonates while type V was the common serotype among non-pregnant adults (Blumberg *et al.*, 1996).

Characterization of GBS also include susceptibility towards antibiotic and it can correlate with distribution of their serotypes which suitable to establish epidemiological studies. Generally, antibiotic susceptibility testing AST is still the one of the most important technique in clinical microbiology field. This is because the confirmatory analysis in some cases is always based on AST's result and summary from AST data is used to drive towards empiric antimicrobial therapy (Doern, 2011).

Yow *et al.*, (1980) suggested both colonization of neonatal GBS and GBS EOD disease can be reduced by the use of intrapartum antibiotic prophylaxis (Yow *et al.*, 1980). Then in 1996, the Centers for Disease Control and Prevention (CDC) had published the guidelines for this prophylaxis and recommended that penicillin or ampicillin is the preferred drug for treating GBS (Centers of Disease Control and Prevention, 1996). Besides, for the women patients who are allergic to penicillin, the use of erythromycin and clindamycin was administered to them (Lin *et al.*, 2000). However, the penicillin-resistance in GBS strains were started to emerge, which has become a concern.

PCR-Random Amplified Polymorphic DNA (RAPD) is one of other molecular techniques which can be used in fingerprinting of GBS bacterial isolates. RAPD is valuable to facilitate the discovery of unique genetic markers in association of bacterial genus and species instead of establishing a DNA sequence diversity among bacterial isolates (Welsh & McClelland, 1990). RAPD acts as an alternative molecular method for typing invasive GBS instead of Pulsed Field Gel Electrophoresis (PFGE), since PFGE is the gold standard for bacterial typing and it generally yields a high amount of strain pattern diversity; also the pattern is consistent. However, PFGE is time and labor consuming which effect the duration of the research study. RAPD procedure was significantly easy to perform and less time consuming. RAPD is robust enough to be considered as a DNA analytical technique for molecular investigations.

After 65 years, GBS had become an important cause of infection in neonates, pregnant women and non-pregnant adults (Farley, 2001). Until now, the reports about GBS infection especially in immunocompromised adults over the years can now be considered as a serious problem. Besides, the information about distribution of GBS infection especially in Malaysia is still limited. From this, one of the objectives of studies is to establish the data about distribution of GBS infection so that it acts as an additional reference for intrapartum prophylaxis and prevention. Therefore, serotyping and AST of GBS isolated from a few hospitals in Klang Valley will provide some interesting data about distribution of GBS in this country.

Besides that, the study also looked into the antibiotic resistance pattern of GBS isolates via AST. This is because there is some probability which high-level intrinsic resistance to many antibiotic classes may be occur. Furthermore, there is possibility that the resistance to penicillin is started to emerge although this drug is the preferred drug for treating GBS as recommended by CDC. In another words, the increasing occurrence of acquired resistance to the first-line drug penicillin will hamper the management of infection. The serious infections caused by pathogenic bacteria which become resistant to commonly used antibiotics used by clinicians have become a major global healthcare problem in the 21<sup>st</sup> century (Alanís *et al.*, 2005). Thus, the antibiotic resistance pattern is important which can provide better prophylaxis against GBS infection.

Furthermore, the study also hopes to give any information of evolution exists between GBS isolates that comes from different area due to dissemination of strains. This analysis can be used as a reference for distribution data of GBS strains. RAPD is used to analyze the genetic diversity of an organism by using random primers (Welsh & McClelland, 1990) and amplify DNA segments at particular template at random. The dendrogram produced from the analysis will explain the dissemination and diversity of GBS isolates according to their clusters.

The objectives of the study are:

General objective is to study distribution of GBS isolated from a few major hospitals in Malaysia.

Specific objectives are;

1. To identify the antimicrobial susceptibility patterns of GBS isolated from clinical samples.
2. To determine the serotype of GBS isolated from clinical samples by using latex agglutination method and multiplex PCR.
3. To determine the molecular fingerprinting of GBS isolated from clinical samples by using RAPD technique.

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