UNIVERSITI PUTRA MALAYSIA

ANTIBIOTIC SUSCEPTIBILITY PROFILES AND GENOTYPIC CHARACTERISTICS OF TETRACYCLINE–RESISTANT Streptococcus pyogenes ISOLATED FROM HOSPITALS IN MALAYSIA

AYA MUKTAR ABD ULATIF

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By

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Abstract of research project presented to the Senate of Universiti Putra Malaysia in partial fulfillment of the requirement for the degree of Master of Science.

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April 2017

Chairman : Associate Professor Rukman Awang Hamat, PhD
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Streptococcus pyogenes (group A streptococcus) is one of the most prevailing human pathogens causing a wide variety of infectious diseases and immunological complications. Tetracyclines are one of the alternative drugs used for its therapy. However, there is an increasing evidence of resistance and clinical failures towards these drugs reported globally. This condition is now becoming a great concern as tetracycline resistance genes can be easily transferred via horizontal gene transfer as well as the spread of clonal strains. Moreover, certain *emm* types and clones are said to be associated with particular diseases. The aims of this study were to determine antibiotic susceptibility profiles and genotypic characteristics of tetracycline resistance among *S. pyogenes* isolates as well as to identify the M-protein by means of *emm*-typing. A total of 42 *S. pyogenes* clinical isolates were obtained from Kuala Lumpur and Serdang hospitals. Phenotypic detection methods were carried out by using Gram-stain, colony morphology on Blood agar, bacitracin and PYR tests. Antibiotic susceptibility testing was done by disc-diffusion method and interpreted according to CLSI guideline. Minimum inhibitory concentration of tetracycline-resistant isolates were conducted by E-test method and interpreted according to CLSI guideline. Tetracycline resistance genes were detected by PCR using established primers. *emm* typing was established by sequencing the PCR products. All isolates were susceptible to penicillin, erythromycin and linezolid. Thirty isolates (71.4%) were resistant to tetracyclines which comprised tetracycline, minocycline, and doxycycline. Of 30 tetracycline-resistant strains, 73.3% of isolates harboured *tet*(M) gene alone while 10%, 10% and 3.3% had a combination of *tet*(M)/*tet*(L), *tet*(M)/*tet*(O)/*tet*(L) and *tet*(M)/*tet*(O) genes, respectively. No tetracycline genes were detected in one resistant isolate (3.3%). GAS from non-invasive specimens exhibited higher number of tetracycline-resistant isolates compared to invasive specimens (42.8% versus 28.5%). A total of 25 different *emm* types were detected. The most frequent *emm* types identified were *emm*63 (9.5%) followed by 7.1% of *emm*18.21, *emm*28.5, *emm*1.0, *emm*89 and *emm*91. No new *emm* types were detected. Eighteen *emm* types (72%) were tetracycline resistance strains and *emm*63 (13.3%) was frequently described among them. *emm*18.21 type (3 isolates) was the most frequently described among invasive isolates. High number of tetracycline-resistant GAS and its tetracycline genes in the present study possesses a great risk of impending outbreaks in hospital and community settings. Antibiotic surveillance system and antibiotic stewardship program should be continuously maintained to overcome this problem in future. High genetic diversity of *emm* types would hinder the development of suitable vaccine candidates. However, a multicentre study is warranted to establish the potential vaccine coverage for our local population.

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

PROFIL KERINTANGAN ANTIBIOTIK DAN CIRI GENOTIP *Streptococcus pyogenes* RENTAN-TETRASIKLIN YANG DIPENCILKAN DARI HOSPITAL DI MALAYSIA

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Streptococcus pyogenes (kumpulan A streptococcus) adalah salah satu daripada patogen manusia yang paling menonjol menyebabkan pelbagai jenis penyakit berjangkit dan komplikasi imunologi. Tetrasiklin adalah salah satu ubat alternatif digunakan untuk rawatan. Walaubagaimanapun, terdapat banyak bukti tentang kerintangan dan kegagalan klinikal terhadap ubat-ubatan ini dilaporkan di seluruh dunia. Keadaan ini telah menjadi satu permasalahan utama kerana gen-gen rintangan tetrasikilin dapat dipindahkan dengan mudahnya melalui pindahan gen horizontal dan juga penyebaran strain klonal. Di samping itu, taipan *emm* dan klon-klon tertentu dikatakan telah dikaitkan dengan penyakit-penyakit yang tertentu. Tujuan kajian ini adalah untuk menentukan profil kecenderungan antibiotik dan ciri-ciri genotip rintangan tetrasiklin antara *S. pyogenes* serta mengenal pasti M-protein diasingkan dengan menggunakan pengetipan *emm*. Sejumlah 42 *S. pyogenes* pencilan klinikal di perolehi dari koleksi bakteria yang sebelumnya di Makmal Mikrobiologi, Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia. Kaedah-kaedah pengesanan fenotip telah dijalankan dengan menggunakan pewarnaan Gram, morfologi koloni pada agar darah, ujian basitrasin dan PYR. Ujian kerentanan antibiotik telah dilakukan dengan kaedah cakera resapan dan ditafsirkan mengikut garis panduan CLSI. Kepekatan perencatan yang minimum bagi pencilan-pencilan rintang tetrasiklin telah dilakukan dengan kaedah E-test dan ditafsirkan mengikut garis panduan CLSI. Gen-gen rintangan tetrasiklin telah dikesan oleh PCR dengan menggunakan primer-primer yang sudah tersedia ada. Pengetipan *emm* telah dicapai melalui penjujukan produk-produk PCR. Semua pencilan-pencilan adalah rentan kepada penisilin, eritromisin dan linezolid. Tiga puluh pencilan (71.4%) adalah rintang kepada tetrasiklins yang terdiri dari tetrasiklin, minosiklin dan doksisiklin. Daripada 30 pencilan rintang tetrasiklin ini, 73.3% pencilan memiliki hanya gen *tet(*M) semata-mata, manakala 10%, 10% dan 3.3% pencilan masing-masing mempunyai campuran gen-gen *tet*(M)/*tet*(L), *tet*(M)/*tet*(O)/*tet*(L) dan *tet*(M)/*tet*(O). Tiada gen rintangan tetrasiklin dikesan pada satu pencilan rintang tetrasiklin (3.3%). GAS yang diperolehi dari spesimen yang tidak invasif mempamerkan bilangan pencilan rintang tetrasiklin yang tinggi berbanding dengan spesimen invasif (42.8% lawan 28.5%). Sebanyak 25 jenis taipan *emm* yang berbeza telah dikesan. Taipan *emm* yang paling kerap dikesan ialah *emm*63 (9.5%) diikuti dengan 7.1% setiap taipan *emm*18.21, *emm*28.5, *emm*1.0, *emm*89 dan *emm*91. Tiada taipan *emm* yang baharu dikesan. Lapan belas taipan *emm* (72%) adalah strain rintang tetrasiklin dan *emm*63 (13.3%) adalah yang paling kerap digambarkan di kalangan taipan *emm* itu. Taipan *emm*18.21 (tiga pencilan) adalah yang paling kerap digambarkan di kalangan pencilan-pencilan invasif. Jumlah yang tinggi bagi GAS rintang tetrasiklin dan gen tetrasiklin dalam kajian ini mempunyai risiko yang besar akan berlakunya wabak dalam persekitaran hospital dan komuniti. Sistem pemantauan antibiotik dan program pengawasan antibiotik perlu terus dikekalkan untuk mengatasi masalah ini pada masa akan datang. Kepelbagaian genetik yang tinggi dalam taipan *emm* akan menghalang pembangunan vaksin yang sesuai. Walau bagaimanapun, satu kajian multisenter adalah wajar untuk mewujudkan perlindungan vaksin yang berpotensi untuk populasi tempatan.

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I certify that a Thesis Examination Committee has met on 27 April 2017 to conduct the final examination of Aya Maktar Abadulatif on her thesis entitled "Antibiotic Susceptibility Profiles and Genotypic Characteristics of Tetracycline-Resistant *Streptococcus pyogenes* Isolated from 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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BHS	β-haemolytic streptococcus
GAS	Group A streptococcus
tet (M)	Tetracycline resistance gene M
tet (L)	Tetracycline resistance gene L
tet (O)	Tetracycline resistance gene O
Bp	Base pair
CO2	Carbon dioxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
E-Test	Epsilometer test
HKL	Hospital Kuala Lumpur
HS	Hospital Serdang
MHA	Muller Hinton agar
MHBA	Muller Hinton blood agar
MICs	Minimum inhibitory concentrations
PCR	Polymerase chain reaction
TBE	Tris-borate EDTA
STSS	Streptococcal toxic shock syndrome
ARF	Acute rheumatic fever
RHD	Rheumatic heart disease
APS	Acute post-streptococcal glomerulonephritis
NF	Necrotising fasciitis
SOF	Serum opacity factor
RAPD	Random amplified polymorphic DNA analysis
MLEE	Multilocus enzyme electrophoresis

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CHAPTER 1

INTRODUCTION

Streptococcus pyogenes (group A β-haemolytic streptococcus) often referred to as group A streptococci (GAS), is one of the most prevalent human pathogens responsible for a wide range of clinical diseases such as acute pharyngitis, streptococcal shock syndrome (STSS), necrotizing fasciitis and skin abscess. Indeed, this infectious pathogen has become an important cause of morbidity and mortality among people all over the world (Friães, Lopes, Melo-Cristino, & Ramirez, 2013). The wide variety of infections that are caused by *S. pyogenes* is due to the expression of a many set of virulence characteristics (Fiedler, Sugareva, Patenge & Kreikemeyer, 2010). However, prompt antimicrobial therapy with administration of penicillin is considered as the first line of therapy and the preferred drug of choice. Alternatively, other drugs such as tetracyclines can be used as treatment for patients who are allergic to penicillin (Rubio-López et al., 2012). Tetracyclines have been used in the treatment of a wide range of GAS infection and other Gram-positive and Gram-negative bacterial infection since 1950s (Roberts, 2003).

The global burden of diseases caused by GAS is enormous and according to WHO, it is ranked as the ninth leading infectious cause of human mortality, with majority of death resulting from invasive infection and rheumatic heart disease (RHD), particularly in non-developing countries. It has been reported that about 18.1 million cases of acute GAS disease with 1.78 million new cases were documented every year (Carapetis, Steer, Mulholland & Weber, 2005). However, in recent years the resistance to tetracycline has attracted the attention of clinicians and scientists all over the world. The increased level of tetracycline resistance among GAS was attributed to the acquisition of *tet* gene and this resistance was associated with mobile genetic elements. Nevertheless, the prevalence rates of tetracycline resistance vary according to geographical area, type of local strains and methods of the study used. In Malaysia, preliminary data indicates high rate (42.9%) of tetracycline resistance among GAS (Kalgo, 2015). However, studies in Spain and Sweden documented 7.3% and 1.3% of tetracycline resistance among their isolates, respectively (Orden, Martinez, López, & Franco. 1996; Strömberg, Schwan & Cars, 1988). In India, high prevalence rate of tetracycline resistance (73%) was reported among GAS isolates in their population. In their study, high level of resistance was attributed to indiscriminate use of antibiotic in the community (Mathur et al., 2014).

The most extensively studied bacterial virulence factor for GAS is M protein. This protein constitutes a significant surface protein component of the bacteria and has been identified to play a very important role in the pathogenesis of the microorganism (Smeesters, Mardulyn, Vergison, Leplae & Van Melderen, 2008). In the late 1990s, serotyping was used to type the M protein by using specific rabbit antisera but now it has been replaced by sequence typing of the 5′ end of the M protein which is known as *emm*-typing (Steer, Law, Matatolu, Beall & Carapetis, 2009).

1.1 Problem statement

Globally, many bacterial diseases continue to infect people and cause significant morbidity and mortality among affected individuals. Group A streptococcus infection is one of major human pathogens causing over 600 million infections annually and is considered one of the major cause of acute respiratory tract infections (Carapetis et al., 2005). However, the increasing level of antibiotic resistance among *S. pyogenes* clinical isolates has emphasized the need for continuous surveillance of antimicrobial resistance patterns (Chen, Kaufisi & Erdem, 2011). Bacteria are capable of acquiring virulent and antimicrobial resistance genes from other strains and this is one of the major factors responsible for the emergence and evolution of resistant pathogenic isolates.

1.2 Significance of the study

The prevalence of antimicrobial resistance among GAS presents a serious challenge due to the burden of treatment cost and prolonged treatment regime. This study was conducted to determine the characteristics of tetracyclines resistance in clinical-acquired *S. pyogenes.* In addition, the distribution of different types of *tet* genes could give new insight on the potential dissemination of these genes among GAS by mobile genetic elements. Molecular epidemiology of GAS by *emm* typing could establish our data for global comparison on the circulating strains in Malaysia as certain *emm* types are believed to be associated with antimicrobial resistance.

1.3 Objectives of the study

1.3.1 General objective:

The aim of this study was to determine the susceptibility patterns of GAS towards tetracyclines and the distribution of tetracycline resistance genes as well as its genetic relatedness by *emm* typing.

1.3.2 Specific objective:

1. To determine the tetracycline susceptibility patterns among *S. pyogenes*.
2. To determine the tetracycline resistance-genes *tet*(M), *tet*(L) and *tet*(O) among *S. pyogenes.*
3. To determine the genetic relatedness of tetracycline resistance strains among GAS isolates with *emm* typing.

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