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**IDENTIFICATION OF VIRULENCE AND ANTIBIOTIC RESISTANT GENE
IN LABORATORY STRAINS *Streptococcus agalactiae***

by

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DECLARATION

"I hereby declare that this thesis is my original work and has not been submitted previously or currently for any degrees at UiTM or any other institutions"

(Muhammad Syafiq Bin Abdul Aziz)

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ABSTRACT

IDENTIFICATION OF VIRULENCE AND ANTIBIOTIC RESISTANT GENE IN LABORATORY STRAINS *Streptococcus agalactiae*

Streptococcus agalactiae or Group B *Streptococcus* (GBS) is a causative agent for serious infection and neonatal sepsis that lead to neonatal morbidity and mortality. Previous study proved that the pathogenesis of *Streptococcus agalactiae* infection is complex and the severity can be predicted by the identification of the virulence factors encoded among others by the *cps* gene cluster coding of the *Streptococcus agalactiae* capsule. However, there is a lack of study done on the laboratory strains *Streptococcus agalactiae*. Study done stated that the laboratory strains are unable to fully represent microorganism that growth in the 'real-world' environment. This study is done to identify the virulence genes and antibiotic resistance genes in *Streptococcus agalactiae* isolates from laboratory reference strains. The laboratory strains *Streptococcus agalactiae* are sub-cultured into two different time frames in order to observe for any genetic drift or variations. The detection of the virulence gene *lmb, cyl*E, *bca, scp*B, and *rib* gene and antibiotic resistant genes *ermB* gene and *tetO* gene are done by using SYBR Green real-time PCR. All the samples from both short term and long term time frame were analyzed for the presence of the virulence and antibiotic resistant genes. The outcomes of the analysis indicate there were no genetic drifts or variants for *lmb, cylE bca, scpB* gene even after a series of passage. The *rib* gene is undetermined for genetic variations or drift since not all the sample expressed the gene. The *ermB* gene and *tetO* gene analysis were undetermined since the results of the analysis are not specific. The comparison between clinical sample and laboratory strains show that the laboratory strains expressed the same virulence genes as the clinical samples. This study suggested that the used of real-time PCR are specific and sensitive methods that could be used to detect the virulence gene of *Streptococcus agalactiae*.

Keywords: *Streptococcus agalactiae*, virulence genes, antibiotic resistant genes, real-time PCR.

CHAPTER 1

INTRODUCTION

1.1 Study background

   Streptococcus agalactiae or Group B *Streptococcus* (GBS) is a causative agent for serious infection and neonatal sepsis that lead to neonatal morbidity and mortality, *Streptococcus agalactiae* also act as an opportunistic agents for infection in pregnant women, adult especially elderly with underlying medical condition (Dutra *et al,* 2014). Infection in neonate is divided into two stages; early-onset and late-onset. Early onset infection occurs within the first week of life while late-onset diseases occur between 1 week and 3 month of age (Eskandarian *et al.*, n.d.). Center of Diseases Control (CDC) in USA are recommending for all the pregnant women at 35 – 37 weeks of gestation to be screen for *Streptococcus agalactiae* colonization to reduce the risk of neonate infection of *Streptococcus agalactiae*. Antimicrobial Prophylaxis that are offered to women with positive colonization of *Streptococcus agalactiae* will lead to more than 70% reduction in early onset GBS infection in neonates (Hannoun *et al.*, 2009).

   There are ten serotype of *Streptococcus agalactiae* which are Ia, Ib, II, III, IV, V, VI, VII, VIII and IX according to their unique polysaccharide capsule (Laczeski, Pegels, Oviedo, Quiroga, & Vergara, 2014) . However, serotypes of *Streptococcus agalactiae* are found to be varied according to the time and also geographical factor. In the USA and Europe, the predominate *Streptococcus agalactiae* serotype are Ia, II, III and V, while serotype Ib and III are found to be the predominate serotype in Japan. Previous study done in Malaysia determine that serotype Ia and VI are the predominate *Streptococcus agalactiae* serotype among pregnant women (Eskandarian *et al.*, n.d.).