MOLECULAR IDENTIFICATION OF VIRULENCE GENES IN LABORATORY STRAINS *Streptococcus pneumoniae*

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DECLARATION

I hereby declare that this thesis is based on my original work. I also declare that this thesis has not previously or concurrently submitted by any other degree students at UiTM or other institutions.

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TABLE OF CONTENTS

ABSTRACT

MOLECULAR IDENTIFICATION OF VIRULENCE GENES IN LABORATORY STRAINS *Streptococcus pneumoniae*

Streptococcus pneumoniae is the main cause of pneumococcal diseases ranging from non-invasive condition of otitis media and sinusitis to more invasive conditions of meningitis and septicemia particularly in children, elderly and immunocompromised. Virulence genes found in *Streptococcus pneumoniae* are *lytA, ply, PsaA, PspA, cbpA, PavA, nanA, nanB, eno,* and *psrP*. There is limited information about the distribution of virulence genes in *Streptococcus pneumoniae* isolates from laboratory strains *Streptococcus pneumoniae* in comparison with clinical strains. Therefore, this study was conducted to detect virulence genes (*lytA, ply, PsaA, PspA, cbpA, PavA, nanA, nanB, eno,* and *psrP*) and to compare the virulence genes distribution in *Streptococcus pneumoniae* from laboratory reference strains with clinical strains. A sample *Streptococcus pneumoniae* (ATCC 6305) from laboratory strains was obtained from Microbiology laboratory, UiTM Puncak Alam and the bacterial DNA was extracted using the boiling method. The detection of *lytA, ply, PsaA, PspA, cbp A, PavA, nanA, nanB, eno,* and *psrP* in *Streptococcus pneumoniae* from laboratory reference strains was done using SYBR Green real-time PCR. In this study, the biochemical and molecular tests confirmed the isolates as *Streptococcus pneumoniae* (ATCC 6305). The real-time PCR showed that all isolates were positive for virulence genes *LytA, Ply, NanB* and *PspA* genes. No amplification was seen for *PsaA, cbpA, PavA, nanA, eno* and *psrP* genes. Hence, this study indicates that, with such genotypic distribution pattern, *Streptococcus pneumoniae* from laboratory strains is a less pathogenicity as only four virulence genes detected compared with virulence gene's presence in the clinical strains. The application of real-time PCR proved a rapid and specific method for the detection of virulence genes in *Streptococcus pneumoniae.*

Keywords: *Streptococcus pneumoniae,* virulence genes, laboratory strains, real-time PCR

CHAPTER 1

INTRODUCTION

1.1 Study background

Streptococcus pneumoniae is gram positive diplococci or can also be in chain of cocci, a very fastidious bacterium that require approximately about 5% of CO_2 at 35°C to 37°C of temperature to grow best. The morphological characteristics of *Streptococcus pneumoniae* are small, grey in colour and mucoid colonies seen on blood agar plate. Besides, *Streptococcus pneumoniae* has one interesting characters on blood agar which the production of zone of alpha-haemolysis or partial haemolysis (green). In addition, optochin sensitivity is done for identification of alpha-hemolytic streptococci as *Streptococcus pneumoniae* while other streptococci strains are optochin- resistant. The bile solubility test is performed for the confirmation of *Streptococcus pneumonia* as only *Streptococcus pneumonia* is bile soluble (Dash, 2013).

Streptococcus pneumoniae is one of the most pathogenic bacterium which remains as the major cause of severe infection such as pneumonia, septicemia, and meningitis. Moreover, bacteraemia, otitis media, sinusitis, and meningitis may become very pathogenic to the children, the elderly and immunocompromised patients (Sakai *et al.,* 2013). It is believed that in most developing countries, previous studies have shown a very high number of young children, which about 1 million who died because of pneumococcal diseases every year. This is in line with a report stated in the United Nations, that the percentage of elderly citizens from aged 60 and above is estimated to increase from 9.9% in 2010 to 23.6% in 2050.