CHAPTER I

INTRODUCTION

Streptococcus pneumoniae are the most important cause of respiratory tract infections including pneumonia, sinusitis, otitis media and bacteremia (1, 2). Since its detection in 1967, penicillin resistance in *S. pneumoniae* has become increasingly prevalent worldwide (3). Macrolides have become good therapeutic choice in the treatment of penicillin-resistant *S. pneumoniae* infections and in penicillin-allergic patients with penumococcal pneumonia (4, 5). However, macrolide resistance in *S. peumoniae* is increasing in many countries and in other parts of the world (6-8).

There are two major mechanisms for macrolide-resistant *S. pneumoniae*. The first is mediated by methylation of highly conserved adenine residues in the peptidyl transferase center of the 23S rRNA. The methylase is encoded by the *erm* gene (erythromycin ribosome methyase) (9-13). This methylation blocks the binding of macrolides, lincosamides and streptogramin B, due to the overlapping binding sites of the drugs (14, 15). Macrolide resistance mediated by *erm* (B) has typically been associated with high-level resistance (MIC_{90} values of $\geq$64 μg/ml) (11). The expression of the *erm* (B) gene causes resistance to macrolides, lincosamide and streptogramin B, designated MLS_B phenotype. In addition, expression of MLS_B resistance can be constitutive ($cMLS_B$) or inducible ($iMLS_B$) (14, 16). The *erm* (B) gene has become more common in macrolide-resistant *S. pneumoniae* in Europe (17-19), South Africa (20, 21) and some countries in Asia such as Hong Kong, Korea and Janpan (22-24).

The second major mechanism of resistance is mediated by an active efflux pump, encoded by the *mef* gene (macrolide efflux). The macrolide efflux removes the drug from bacterial protoplasm by a proton motive force-driven transporter (25). The Mef protein has been characterized by lower-level resistance (MIC range of 1-16 μg/ml) (26, 27). The expression of the *mef* gene produces resistance only to 14- and 15-membered macrolide, designated M phenotype (12, 25). The M phenotype of *S. pneumoniae* predominates in North America (28-35). However, some European countries such as Germany and Austria have reported an increasing incidence of the

efflux mechanism (36-39). There are two subclasses of the *mef* gene : *mef* (A), originally found in *Streptococcus pyogenes*, and *mef* (E), originally found in *S. pneumoniae*. The *mef* (A) and *mef* (E) genes are 90% identical at the nucleotide level but are characterized by major genetic differences (26, 40-42). Both *mef* (A) and *mef* (E) are now found in *S. pneumoniae* (43, 44) and are known to be transferable (45). In addition, pneumococcal resistance to macrolides may also result from ribosomal mutations in gene encoding 23S rRNA and ribosomal proteins L4 and L22 (37, 46). However, reports to date of ribosomal mutations among macrolide resistance in *S. penumoniae* are rare (47, 48).

Genetic elements carrying the *mef* (A) and *mef* (E) genes were recently characterized in *S. pneumoniae*. The *mef* (A) carrying element is 7.2-kb and located on transposon (Tn*1207.1*) that contains eight open reading frames (ORFs) (43). The *mef* (E) carrying transposon element, designated MEGA (macrolide efflux genetic assembly), is approximately 5.5-kb and contains five ORFs (49, 50). The sequence instantly 3' of the *mef* (A/E) gene are a 1,464-bp ORF, was designated *mel* gene. The *mel* gene shares approximately 36.2% identity with the macrolide and streptogramin B resistance protein A (*msrA*) gene in *Staphylococcus epidermidis* that putatively encodes an ATP-binding cassette (ABC) transporter protein (49, 51). However, the presence of *mel* gene among macrolide-resistant *S. pneumoniae* are rarely been identified, whereas *mef* gene had been reported worldwide.

In *S. pneumoniae*, *mef* (E) and *mel* are cotranscribed as an operon and are predicted to be a dual efflux pump (49, 52). The size of the intergenic region between *mef* (E) and *mel* genes appeared approximately 119 bp. However, this intergenic region was found a 99-bp deletion in M-phenotype *S. pneumoniae* isolates by two studies by Weierzbowski A. K. *et al.*, 2005 (53) ; Gay K. *et al.*, 2001 (49). Sequencing analysis of the upstream *mef* (E) was reported by Weierzbowski A. K. *et al.*, in 2005. It was found that there were a T78G substitution, A75T substitution, T52G substitution and T30C substitution, T60 deletion and 16-bp deletion at position -153 bp of the putative start codon. Nevertheless, there is no data on the relationship between mutations in the upstream region of *mef* gene and the MIC level of macrolide-resistant *S. pneumoniae*.

The purpose of this study was to investigate the prevalence of macrolide resistance and characterize the resistance gene including *erm* (B), *mef* and *mel* genes in *S. pneumoniae* isolates from patients at King Chulalongkorn Memorial Hospital between 2003-2007.