CHAPTER VII

CONCLUSION

Macrolides are the drugs of choice in the treatment of penicillin-resistant *S. pneumoniae* infections and in penicillin-allergic patients with penumococcal pneumonia. Macrolide-resistant *S. pneumoniae* has increased in many countries over the wolrld. A total of 385 *S. pneumoniae* isolates were collected from patients at King Chulalongkorn Memorial Hospital, Bangkok during January 2003 to December 2007. The prevalence of macrolide resistance was 54.02% for erythromycin and 53.76% for clarithromycin. MIC_{50} and MIC_{90} of macrolides were 2 μg/ml and >512 μg/ml. Prevalence of clindamycin resistance was 25.20%. MIC_{50} and MIC_{90} of clindamycin were 0.125 μg/ml and >512 μg/ml. Macrolide resistance phenotype was identified by double disc diffusion test using erythromycin and clindamycin. Among the 208 erythromycin-resistant isolates, 96(46.15%) were resistant to macrolide and clindamycin and showed $cMLS_B$ phenotype, whereas 112(53.85%) were resistant to macrolides but remained susceptible to clindamycin and exhibited the M phenotype. The $iMLS_B$ phenotype were not detected. Detection of macrolide resistance genes in *S. pneumoniae* was investigated by multiplex PCR. The *erm* (B) gene was found in 95 isolates (45.67%) exhibited high level MIC and the *mef* gene was identified in 112 isolates (53.85%) exhibited low level MIC. One isolate (0.48%) carried both *mef* and *erm* (B) genes exhibited high level MIC. Detection of *mef* (A/E) type genes was investigated by PCR-RFLP. The *mef* (E) gene was detected in all M phenotype isolates. The erythromycin MIC of 112 M-phenotype *S. pneumoniae* were decreased 6-9 fold in the presence CCCP, an efflux pump inhibitor, confirming the presence of an efflux mechanism. DNA sequence analysis of M-phenotype *S. pneumoniae* (MIC 1-16 μg/ml) revealed a 1,218-bp ORF of entire *mef* (E) gene, encoding 405 amino acids and 1,464-bp ORF of entire *mel* gene, encoding 487 amino acids. All 10 sequences of entire *mef* and *mel* genes were identical to each other at the nucleotide and amino acid levels and also identical with the *mef* (E) published sequences in GenBank. Analysis of a 630 bp upstream region of *mef* (E) gene showed 23 nucleotide changes ; T to C at postion

-31, T to G at position -54, T deletion at position -63, A to T at position -78, T to G at position -81, A to G at position -82, T to A at position -345 and 16 bp deletion at position -155 upstream of *mef* (E) gene in all 10 M-phenotype *S. pneumoniae*. Four isolates carrying T-345A substitution had the MIC range of 2-16 μg/ml whereas the other 6 isolates with no mutation in this position had the MIC range of 1-4 μg/ml. The results demonstrated that mutation at T-345A may be associated with increased erythromycin MIC in M-phenotype *S. pneumoniae*.