CHAPTER VI

DISCUSSIONS

This study should help to demonstrate the use of *emm* typing besides standard serological methods as an accurate method to characterize variability of GGS and GCS. However, *emm*-typing system can be applied to GGS isolates better than GCS isolates. In this study, *emm* gene from some GCS isolates can not be amplified by PCR method from CDC protocol. It is possible that *emm* gene of GCS has polymorphism at primer site. Different sets of primers were used to prove this problem. MF2 and MR1 from publications by A. Podbielski and colleagues were used in this study. However, MF2 and MR1 still can not amplify *emm* gene from those GCS isolates. Another most likely hypothesis is that some GCS may not have *emm* gene. However, this hypothesis has to be proved by hybridization method using conserved *emm* sequences, which was not done in this study.

In summary, sequence analysis of the 60 GGS isolates used in this study revealed 28 (47%) different *emm* types, which are 6 (18%) *emm* variant types and 10 (26%) novel sequence *emm* types that were not identifiable with previously published *emm* sequences. From 52 GCS isolates, 24 isolates were amplified and sequenced. In this group, there are 12 (50%) different *emm* types, which is 7 (38%) novel sequence *emm* types that were not identifiable with previously published *emm* sequences. This finding shows the diversity and unique population of GGS and GCS strains in Thailand. However, it is also possible that the unique *emm* sequence types is due to the limited study of *emm* typing of GGS and GCS. When the *emm* types from this study were compared to the collection of CDC and GenBank database. Twenty-five *emm* types found in this study were similar to CDC database in which most of the samples were isolated from United State. When the *emm* types of this study were compared to the study of *emm* types from Schnitzler and his colleges study in 1995. The sample of Schnitzler study was isolated from Minneapolis and Toledo. Four *emm* types of this

study were similar to Schnitzler and his colleges study. Namely, *ST480, ST485, ST643*, and *ST653.*

Interestingly, the same *emm* types can associate with both GGS and GCS specimens. For example, *H46A* type was found both from GGS and GCS isolates. In addition, some *emm* sequence types of GGS isolates are similar to the *emm* types of GAS isolates. For example, *emm23 variant* type isolated from invasive GGS specimen that has distinct *emm* sequence but closely related to *emm* sequence of *emm23* from GAS with homology ~ 95% (Figure 13E). *NSGemm3.1 novel emm* type was isolated from non-invasive GGS specimen, which is 87% homology to *emm3.1* from GAS (Figure 14E) and *NSGemm100.1 novel emm* type was isolated from invasive GGS specimen, which is 89% homology to *emm100.1* from GAS (Figure 14C). This study confirms the evidence of interspecies transfer of *emm* genes (horizontal gene transfer) between GAS and GGS. In 1992, Simpson and his colleague found the evidence of horizontal transfer and recombination event involving movement of part or all of *emm* gene from *emm12* GAS donor to GGS recipient.

In the present time, the vaccination against M protein of GAS is very important. Because GAS is a major pathogen of human and can cause severe infections (STSS, AGN, and ARF). Currently, antibody to M protein is the most important factor to prevent infection. However, M proteins are highly heterologous, the development of vaccine of GAS is basing on the database of *emm* sequence types. The effective vaccine should compose of multiple types of M protein (*emm*) gene that important in causing diseases. In addition, from the evidence of horizontal gene transfer among *Streptococcus*, the *emm* types database of GCS and GGS were also needed to support the information for the vaccine development to GAS.

The associations of *emm* types with invasive and non-invasive specimens from GGS isolates were also compared in this study. The distributions of *emm* types seem to be different between invasive and non-invasive groups. Although the difference are not statistically significance by chi-square analysis. For example, *STC6979* (7 isolates,

12%) was found more frequently in invasive groups and *STC5345* (6 isolates, 10%) was found more frequently in non-invasive GGS as shown in table 15. The lack of association might be because the sample size is not big enough. However, these data might be useful in the follow up process of patients who are infected with isolate with particular *emm* type that associates with invasive infection. Further studies are needed to identify *emm* types from GCS and GGS that associates with severe infections such as scarlet fever, streptococcal toxic shock syndrome, necrotizing fascitis, rheumatic fever and acute glomerulonephritis.

In conclusion, M protein (*emm*) typing is a useful tool for conducting epidemiological studies of streptococcal infections, particularly in an area where severe streptococcal infections are commonly found. Information of *emm* type from GCS and GGS in Thailand allows not only monitoring of streptococcal carriage within regions of endemicity but also identification of types of circulating streptococci. This study provides an addition result to the *emm* database of GAS, which will be useful for developing a vaccine for streptococcal infection and rheumatic fever, which is an endemic disease in Thailand.