CHAPTER VI

DISCUSSION

Pulsed-field gel electrophoresis (PFGE) was used to perform genotyping of 135 *P. aeruginosa* isolates from colonized patients in the RCU and the TICU and from the sputum of the patients with respiratory tract infection in the other wards. This technique discriminated all the isolates into 72 different pulsotypes. There was only 5 isolates which was considered to be in the untype group. These isolates possibly produced a large amount of the endonuclease enzyme which could digested the restricted fragments of the chromosomal DNA and left the smeared band on the gel (Goering, personal communication) after the study was performed by PFGE. The PFGE patterns demonstrated considerable diversity among all *P. aeruginosa* 135 isolates according to the interpretation procedures by Tenover et al (15). In fact this high discriminatory power of PFGE techniques has already been demonstrated by the other investigators including Poh et al (129) who successfully resolved 41 different pulsotypes among 44 clinical isolates, and Kersulyte et al (130) who distinguished 17 different types among the 43 isolates from Cystic fibrosis patients. PFGE has been shown to be valuable in the molecular typing because the difference and identity were already recognized among all the isolates tested. Both the number of DNA bands and the case of estimation of band molecular sizes by inspection of gel photograph permitted the unambiguous interpretation. Gel-to-gel comparison of the isolate types, which were necessary for determining the number of genotypes within species in the two ICUs, showed no problematic because of the use of a molecular-size marker and a control strain for each gel and of the identical conditions for electrophoresis in each typing tests. Most of the isolates, which were tested repeatedly overtime, always yielded the same number of bands of the same, estimated molecular size. The

reproducibility of these genotyping methods has been shown in many prior studies. This finding was similar to the study by Talon D. et al (104) which demonstrated the use of pulsed-field gel electrophoresis as an epidemiological tool during an outbreak of *P. aeruginosa* lung infections in an intensive care unit. They showed that the total DNA profiles obtained by PFGE of the appropriate restriction enzyme digests gave excellent typeability, as all strains yield a DNA profile and present very good stability. The reproducibility was very good in triplicate experiments, and including a reference strain facilitated comparison of different gels. Conclusively, the discriminatory power appeared good.

Through the combination of the colonization prevalence, the infection surveillance in patients with colonization and the pulsotyping, the epidemiological study of *P. aerginosa* was also performed. It was shown that there was no different in the prevalence of *P. aeruginosa* colonization in throat of the patients in the TICU and the RCU which were between 10-14%. This was quite agreed with the result from previous study done by Chetchotisakd P. et al. who showed that the prevalence of *P. aeruginosa* in Indiana hospital center was about 8%.

In this study, it was noticed that the number of the patients who were admitted in the TICU had much higher turn over rate than those of the patients who were admitted in the RCU because 147 patients from the TICU were included in the study while only 82 patients were from the RCU during the 7 months. Most of the patients who were admitted in the RCU were transferred from the other wards and having the chronic illness (78.1%) while most of the patients who were admitted in the TICU were new cases. This could mean that diseases of the patients in the RCU are more serious and chronic. However, length of time before the first acquired *P. aeruginosa* colonization in the RCU patients was 10 days (range 4-14 days) while it was 12 days (range 4-24 days) for the TICU patients. According to this result, it was showed that there seemed to be no different in the time for *P. aeruginosa* colonization acquisition in the patients between the two wards. However this result was slightly different from

the result obtained by Griffith et al. (131) who showed that the oncology patients at Michael Reese Hospital, University of Chicago (Illinois) had average length of stay in oncology wards for 8 day before the first colonization was detected in their throat.

The results obtained from this study showed that the throat colonization with *P. aeruginosa* occurred as early as 4 days and the latest was various from 14-24 days. The wild range of acquired colonization may depend on the severity of the illness in each individual patient. To prevent or minimize the prevalence of *P. aeruginosa* throat colonization, a good oral hygiene should be considered.

Among the 25 RCU patients with tracheostomy, only 3 patients had tracheostomy wound infection with *P.aeruginosa*. Two patients with the same pulsotypes had the *P. aeruginosa* throat colonization as well as infected wound. The organisms were isolated from both sites on the same day suggesting that organism might transfer from throat to wound. Another patient was infected with different strain (pulsotype) of *P. aeruginosa* from that colonized in his throat. *P. aeruginosa* was isolated from wound 5 days after the isolation from her throat. This indicated that such patient did not get infection from the colonized strain. In the TICU, only one patient had *P. aeruginosa* infected wound. The isolate was in the same pulsotype as the colonized organism and was isolated on the same day.

The total 27 patients from both RCU and TICU who were colonized with *P. aeruginosa*, only 4 patients developed *P. aeruginosa* pulmonary infection. Three of these patients were infected by *P. aeruginosa* in the same pulsotypes as those found in throat. Thus, it was shown that patients were more likely to be infected by their own organisms or it could mean that *P. aeruginosa* in respiratory tract infections were the endogenous infection. In addition, patients could be infected by more than one pulsotype in each individual during hospitallization. However, the number of the patients who got infection after colonization by *P. aeruginosa* in this study was low (10-14%) and the prevalence of infected cases during 7 months period of the study were not high enough to obtain conclusive evidence that the colonized patients were

more prone to be infected with *P. aeruginosa* than patients who did not have *P. aeruginosa* colonization. In order to verify this, the pulsotype study of *P. aeruginosa* that colonized in the patients should be performed continuously to see whether there was any change of types of these strains and the period of study should be longer. However, there were various studies indicating that the chronic patients were tended to carry or be infected by more than one strain of *P. aeruginosa*, Boukadida J. at al (105), showed that more than one strain of *P. aeruginosa* could colonized in one patient. He reported that each of the 20 patients which were first colonized by a single strain, then it was rapidly replaced by another remained dominant in the patient's sputum for 15 months.

In this study, similar result was obtained, when the sputum from the same infected patients were collected more than one time. *P. aeruginosa* with different pulsotypes were isolated from the same patient. The emergence of new strains could possibly be associated with the use of antibiotic therapy, which failed to eradicate *P. aeruginosa* strains from the sputum the patients. These finding was consistent with the result obtained by Kersulyte et al (130) who showed that a CF patient could carry more than one strain of *P. aeruginosa* and could carry a given strain for long periods of time. They also pointed out that the *P. aeruginosa* could evolve by changes in drug resistant or other phenotyping traits during long-term colonization.

Among the 12 patients with *P. aeruginosa* colonization in the RCU, 91.7% had been receiving antibiotic prophylaxis including aminoglycosides and β-lactams. Most patients were colonized with *P. aeruginosa* which were resistant to the β-lactams. Similar result was observed in the TICU, all of 15 patients who had *P. aeruginosa* colonization reveived the antibiotic administration including aminoglycosides and β-lactams. Again, most of the *P. aeruginosa* isolated from these patients were resistant to the β-lactam. Eventhough, the antimicrobial agents were administered in most of the patients either for the prophylaxis or the treatment of infections at the other sites, it could not prevent *P. aeruginosa* colonization in the patients. Therefor, there was no

strong evidence that the antimicrobial agents were associated with *P. aeruginosa* colonization in the patients.

For the environmental source, this study has shown that the all of the sinks in both the RCU and the TICU were positive for *P. aeruginosa* at least once during the 7 month of the specimen collection. This finding agreed with the study by Groundmann H. et al (132) which reported that *P. aeruignosa* could be isolated from all sinks in Neonatal Intensive Care Unit. However, after the genotyping study was performed, it was clearly shown that the pulsotype of the strains recovered from the sink was different from the strains that were isolated from the patients in the same unit. This indicated that the environment in the unit, particularly the sinks did not play significant role as the reservoir of *P. aeruginosa.*

Another possible source of *P. aerginosa* is water from a respirator. Eventhough the water from respirator is clean but not sterile and could be one of *P. aeruginosa* source. The results from this study showed that among 167 patients who were on the respirators, in all care unit at Siriraj Hospital only one patient (0.6%) had *P. aeruginosa* positive in the water. This also eliminated the possible role of such water from being the source of the organism.

For antibiogram typing, this technique was able to type all the isolates into 30 different patterns. This typing method had been shown to have very low discriminatory power because *P. aeruginosa* isolates were all multiple resistant organisms and there was no correlation between pulsotypes and antibiogram patterns. This was agreed with the results shown by many investigators including Bingen et al (16) who provided the evidence that antibiogram had relatively limited utility in epidemiological study because antibiotic resistance is affected by extraordinary selective pressure in the hospital. However, the result from the susceptibility test showed that the isolates from sputum were more resistant than those from colonization. This could be explained that the patients who were infected with *P. aeruginosa* in the lower respiratory tract were on antimicrobial treatment for a long period of time which led to the increasing

resistant due to genomic mutation(133). Piperacillin, ceftazidime, imipenem, gentamicin, amikacin, netilmicin and ciprofloxacin were shown to be appropriate in the treatment of *P. aeruginosa* infection because more than 70% of the isolates were susceptible to all these agents.

Finally, by the application of genotyping by PFGE, this study showed the suggestive evidences that there were a number of different strains colonizing the patients in RCU and TICU trauma at Siriraj Hospital. Each patient seemed to be colonized with his own individually distinct strain. Eventhough, each patient can carry more than one strain, there seemed to be no outbreak according to *P. aeruginosa* during the time of study. In infected patients, source of causative agents may come from the organism that colonized in the patent's throat so called endogenous infection. Environment was not the significant source of *P. aeruginosa* in both patients' units.