CHAPTER V

DISCUSSION

I. Incidence of *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *S. aureus* (MRSA)

Nasal cavity is one of the natural permanent habitats of *S. aureus* in men. Range of incidence of *S. aureus* nasal carriers in the healthy (non-medical) personnel was shown to be 10% to 50% (Shoop, 1991 and Volk, 1982) while the incidence of coagulase-negative staphylococci was about 90% (Sommers, 1975). The results in this study showed the similar evidence that there was 12.2% of the healthy (non-medical personnel) Thai personnel who carried coagulase-positive staphylococci in nares and 66.0% of the population carried coagulase-negative staphylococci. In addition, it was also found that there was much lower incidence of *S. aureus* in nasal cavities of non-medical personnel than that of medical personnel. Eventhough, *S. aureus* could be recovered from nasal cavities of both groups of healthy personnel, but all the isolates from the non-medical personnel group were shown to be susceptible to methicillin while 20% of *S. aureus* isolated from the medical personnel group was MRSA. However, other organisms other than staphylococci including gram-negative rods were also

observed in both groups.

The incidence of *S. aureus* was higher in medical personnel than non-medical personnel and MRSA was found only in this study population. In order to investigate the route of bacterial transmission, the study on the incidence of the organism was performed at both nasal cavities and hands of the medical personnel. It was found that the incidence of hand carrier of *S. aureus* was as high as of nasal carrier in the medical personnel. Among the 51 medical personnel who carried *S. aureus*, 7.8% (4 out of 51) of them carried MRSA. Dickson and Czurylo (1988) showed that hand of medical personnel was the transient habitat of *S. aureus* and was one of the routes of transmission of the infecting organism(s) to patients. As the result of this study, conclusive evidence could be drawn that medical personnel was the carrier of *S. aureus* and MRSA. These infecting organism could possibly be transmitted from medical personnel to patients and environment via hands.

Among the patients with wound infections, it was further divided into 2 groups: 1) the burn patients (admitted cases) and 2) the patients who attended at out patient department (non-admitted cases). There was much lower number of burn patients as compared to that of another group of patients. This could be explained by the fact that almost all of the burn patients were in serious conditions and had to stay for longer period than the other patients. On the other hand, the discharge rate of burn

patients was very low. During the one and a half years of study, only 51 burn patients could be included in this study.

The incidence of coagulase-positive staphylococci in burn patients was 74.5% which was almost twice as much of that in patients with wound infection (46.9%). Of the *S. aureus* isolates, a number of MRSA isolation from the burn patients was also shown to be much more higher than that of the other patients. This confirmed that the incidence of MRSA infection was much higher in the burn patients than in the other group of patients.

The matter of fact, it is true that admitted patients were prone to nosocomial infections, including burn patients who mostly had to stay in hospital longer period of time. As the result, it was found that the hospital environment was full of infectious MRSA reservoirs including medical personnel. The patients were debilitated so they were very susceptible to all type of infections. Therefore, many kinds of antibiotics were administered to them for the treatment and prevention of the opportunistic infections. These may cause the increase in the number of resistant mutants including MRSA. The data obtained provided somewhat very important evidence that 76.3% of the burn patients who received any of the previous antibiotic treatment carried MRSA whereas only 46.2% of the patients who had not received any antibiotic treatment carried MRSA. This might be explained by the fact that methicillin-sensitive *S. aureus* (MSSA) had been killed and only rare

cells expressed the resistance traits could grow in the presence of high concentration of drug.

II. Antimicrobial susceptibility pattern of *S. aureus* and MRSA

The main purpose of this part of the study was to determine whether antibiogram could be used as one of the epidemiologic markers. Eventhough, antimicrobial susceptibility testing had been recommended by Mulligan and Arbeit (1991) to be used as an phenotypic technique in epidemiologic study of MRSA, the result provided in this study showed that this could not possibly be so. Susceptibility pattern of 116 MRSA isolates to 18 antimicrobial agents were too much variable that there were 28 different antibiograms observed. In addition, in later study, it has been shown that the strains with similar antibiogram had different plasmid profiles. Thus, it was indicated that antibiogram should not be an appropriate tool for the epidemiologic study on MRSA.

However, the result of antimicrobial susceptibility pattern of *S. aureus* and MRSA provided some useful informations for the choice of therapeutic drug. All of *S. aureus* and MRSA isolates were susceptible to vancomycin. Few other antimicrobial agents had been shown to be the second drugs of choice for MRSA infections; these included clindamycin, neomycin and trimethoprim-sulfamethoxazole.

About 70 to 80% of the isolates were susceptible to these agents. This part of results was very useful for the treatment of *S. aureus* and MRSA infections because it suggested that alternative drugs could be used beside vancomycin which was expensive and could be administered only in the form of injection.

This was agreeable with data from previous studies which showed that vancomycin was efficacy against MRSA for the treatment of serious infections, such as endocarditis, even when other therapies have failed (Sorrell *et al.*, 1982 and Watanakunakorn, 1992). Resistance to vancomycin *in vitro* has not been described for *S. aureus* . Vancomycin inhibited ribonucleic acid and cell wall synthesis and had lethal membrane effect (Cook and Farrar 1978).

Miller *et al* (1960) reported that clindamycin was the aminoglycoside that remained sensitivity to MRSA. Therefore, the results from this study showed that 69.8% of the isolates were sensitive to this agent. However, many investigators informed that resistance could emerge when clindamycin was used as single agent, so it should be used in combination with another drug, preferably vancomycin.

For trimethroprim-sulfamethoxazole, it was shown that 38.3% of MRSA isolates were sensitive to this agent. Therefore, this agent might not be sufficient to be used as alternative to vancomycin. However, many investigators included Elwell *et al.*(1989) and Ardati *et al.*(1989) had

suggested the use of trimethroprim-sulfamethoxazole in serious infections caused by either methicillin-resistant or -susceptible staphylococci, such as cerebrospinal fluid shunt infections and meningitis. Trimethoprim/ sulfamethoxazole in combination with rifampin had been used to eradicate the nasal carriage of resistant staphylococci in nosocomial epidemics. The role of this combination for serious infections has not been defined, but it may be another alternative to vancomycin. Rifampin should not be used alone because resistance can emerge on therapy (Ward *et al.*, 1981)

In spite the fact that none of MRSA was sensitive to penicillin, ampicillin, methicillin, nafcillin and oxacillin, some of these agents had been used as the tools for screening MRSA by paper disk susceptibility technique. It was reported that oxacillin (1-μg/disk), nafcillin (1 μg/disk) and methicillin (5 μg/disk) have been successfully used to detect MRSA (Condron *et al.*, 1986, Boyce, 1984 and Barry and Badal, 1977). Oxacillin has been more preferable by most authorities than other agents because it was the best standardizing agent for the disk diffusion method, the most stable, and perhaps the most reliable. Nafcillin would give the unreliable results when blood was present in the medium. The relative instability of methicillin disk in storage makes it useless desirable (Chambers, 1988). In this study, it was shown that methicillin, nafcillin and oxacillin provided very similar effect against all test

organisms. Therefore, it was supportive that oxacillin could be used for MRSA screening. The other beta-lactam antibiotics had not been recommended to use as MRSA detectors. The failure in the use of cephalosporin disks particularly cephalothin and cephamandole to detect MRSA had been reported (Barry and Badal, 1977). Although, Boyce, 1984 had tried to improve the testing conditions including the use of a 48 hour incubation period, the addition of 5% NaCl to the agar, or the incubation at 30°C, there were still up to 40% of resistant strains missed if cephalosporin disks are used. As a corollary , strains that was resistant to oxacillin, nafcillin, or methicillin, but susceptible to cephalosporins, should be considered resistant (Chambers, 1988).

All of MRSA isolates in this study produced beta-lactamase. The resistant enzyme production by the organisms might be the reason for the high minimum inhibitory concentration (MICs) of methicillin against all these MRSA isolates (>256 μg/ml). Normally, *S. aureus* were considered to be resistant when the MIC of methicillin was 12.5 μg/ml or greater after 24 hour incubation at 37°C (Parker and Hewitt, 1970).

III. Plasmid profile of MRSA in the study populations

The results from the study on plasmid profile analysis of 116 MRSA isolated from 152 patients (51 burn patients and 101 patients with other wound infections) and 115 medical-personnel showed that there were 16 different profiles (including profile 16 which contained no plasmid). Eventhough, MRSA with different plasmid profiles were found in the study populations from different hospitals, the strains with similar profiles were isolated from wounds, nasal cavities and hands of both burn patients and medical personnel in the same hospital.

The most common plasmid profile was profile 5 and the most common size of plasmid found in all profile was 3.1 Kb which was found in 11 out of 16 plasmid profilse. MRSA with plasmid profile 5 was the most common isolate found in burn patients from Siriraj Hospital while profile 9 and profile 3 were other common profiles in this group of population. These three plasmid profiles were also found both in nasal cavities and on hands of medical personnel from this hospital. For the burn patients from Police General Hospital, MRSA with plasmid profile 9 was the most common isolates while only few patients were infected with MRSA profile 1. There were only 2 burn patients from Bhumiphol Adulyadej Hospital who carried and infected with MRSA. These two strains were MRSA profile 1. From the study on burn patients in these three different governmental hospitals, it could be summarized that the most propable

causative agent for infections in burn patients should be MRSA with plasmid profiles 5, 9, 3 and 1, accordingly (Only 2 burn patients were infected with the other profiles included profile 8 and 15).

For the patients with other type of wound infections (all patients were non-admitted cases who came to attend the Out Patient Department (OPD) of Siriraj Hospital), all MRSA isolated from this study population were shown to have similar plasmid profiles as those found in burn patients. However, there was much lower incidence of MRSA infection in this group of patients. From the results obtained in this part of study, the question could have been raised that whether MRSA strains with these four different plasmid profiles (profile 5, 9, 3 and 1) should be consider as virulent MRSA strains. Further investigation should be performed in order to answer this question.

There were many reasons to explain that why there was a higher incidence of MRSA infection in burn patients than that in another group of patients, eventhough it was shown that all isolates were in the similar plasmid profile. This included the longer period of hospital admittion in case of burn patients and the bacterial reservoir which could be found only in the hospital (MRSA could not be isolated from non-medical personnel).

From this study, it was indicated that MRSA infections was nosocomial infection and MRSA transmission occurred from medical personnel to patients via hand

contact and could occurred from infected patients to newly admitted cases.

For the epidemiologic study, it was shown that there was no correlation between plasmid profiles and antimicrobial susceptibility patterns (antibiograms), because MRSA with the same plasmid profiles were shown to have various different antibiograms. This indicated the limitation of antibiogram in the epidemiologic study. In contrast, it was clear that the plasmid profile analysis performed in this study was potentially useful to be used as the epidemiologic marker for MRSA. In this study, the reproducibility of the technique was shown when similar profiles were obtained every time that experiment was repeated (more than twice). This method allowed the reliable extraction of plasmid DNA from colonies of MRSA within about 5 hours. It required no special expertise or equipments and no particularly costly reagents. Another advantage was that the chromosomal DNA were not extracted in sufficient quantities to obscure plasmid DNA fragments after electrophoresis by this method.

Eventhough, there were some limitations of this method for MRSA which carried no or a few plasmids which might be unable to be typed but in this study there was no such as problem because there were only one strain which contained one plasmid (profile 15) and one strain with no plasmid (profile 16). Therefore, it was suggested that

further study using restriction enzyme to digest all the plasmid DNA isolated might be used as the more sensitive procedure for strain identification, particularly when no or only a few plasmids were present.