CHAPTER I

INTRODUCTION

Pseudomonas aeruginosa occupies multiple ecologic niches in nature by virtue of its minimal growth requirements and its ability to produce a large number of extracellular protective and toxic substances(1). These organisms can survive and replicate within the hospital environment, where they colonize sinks (2), hospital distilled water systems (3), and even disinfectant (4). *P. aeruginosa* bacterimia is associated with high mortality in humans, particularly in patients with limited immunocompetence such as those who had severe underlying disease or received cytotoxic chemotherapy as well as premature neonates (5,6). Hospital-acquired infection represents a major source of morbidity and mortality for hospital patients. Among the Gram-negative bacilli that cause nosocomial infection, *P. aeruginosa* has the unique ability to infect a wide variety of animal and plant hosts, and is associated with infections in patient with burns, cystic fibrosis, neutropenia and traumatic wounds, together with the respiratory tract infection (7).

Nosocomial infections represent an important public health problem in the world today. The nation wide rate of nosocomial infection in the USA was estimated to be 5.7 nosocomial infections/100 admissions to acute care hospitals between 1975 and 1976; this is more than the number of hospital admission for either cancer or accidents, and at least four times greater than the number of admissions for acute myocardial infraction (8). Infections of the urinary tract, wound and respiratory systems are the commonest types of nosocomial infections. The bacterial isolates from infected patients in ICU were equally divided between Gram-negative and Gram-positive species. The commonly reported bacteria were *P. aeruginosa* 25%. This finding agrees with the data from the National Nosocomial Infections Surveillance

(NNIS)(9), which showed that the most frequent pathogens isolated from nosocomial infections in ICU(s) (n = 3617 infections, October 1950 to May 1988) were *P. aeruginosa* (13.4%) and also consistent with the report on the isolation of the pathogenic bacteria in Siriraj Hospital, Thailand during 1992 and 1996 which showed that *P. aruginosa* was the most frequently isolated from sputum every year.

P. aeruginosa are notoriously resistant to a large number of commonly used antimicrobial agents; they are usually susceptible to aminoglycosides, the drug of choice(10). Nosocomially acquired *P. aeruginosa* isolates tend to be more resistant to antimicrobial agents than community-acquired strains and frequently displaying resistance to multiple classes of antimicrobial agents (11). The current use of broad-spectrum antibiotics and of aggressive diagnostic and therapeutic techniques, along with the large number of hospitallized patients, contribute to the development of epidemics, which may pass unnoticed if the surveillance system is not sufficiently well developed(12). This can be the case with infections caused by multiply resistant strains of common organisms, in patients with a low infection risk. There was a potential for outbreaks of infection in other areas of the hospital, via the colonized patients who were transferred to the wards from ICU (13). Thus the source of gentamicin, ciprofloxacin resistant *P. aeruginosa* was pursued vigorously to limit potentially disastrous consequences in terms of patient morbidity and mortality and waste of resources such expensive antibiotics.

Various conventional typing systems have been extensively used to study the epidemiology of *P. aeruginosa*(14). Serotyping is by far the most widely employed method of typing of *P. aeruginosa* as well as the pyocin typing a close second. Many laboratories perform pyocin typing which is used in combination with serotyping to obtain greater differentiation between strains of the same serotype. However, the reproducibility of pyocin typing for cystic fibrosis (CF) isolates has been repeatedly shown to be low. Therefore, the usefulness of serotyping was also shown to be limited by the high frequency of both non-typeable and polyagglutinable strains from CF

patients(11). Phage typing is restricted to the national reference centers because of the complication of the system. This method does allow fairly detailed strain identification for approximately 85% of clinical isolate (11). Precise typing methods that can clearly differentiate strains are needed for epidemiological studies of *P. aeruginosa* infections. Identification based on conventional typing methods can be misleading as strains may undergo phenotypic changes. Eventhough the antibiotic susceptibility patterns appears to be more discriminative than some genotypic methods, the technique has still relatively limited utility in epidemiological study because antibiotic resistance is affected by extraordinary selective pressure in contemporary hospitals(16).

During the last decade, traditional methods of strain typing, such as bacteriophage typing and serotyping, have been supplemented or replaced in many laboratories with newer molecular methods, such as multilocus enzyme electrophoresis (MLEE), plasmid profile analysis, restriction endonuclease analysis (REA), ribotyping, PCR-based methods, and analysis of chromosomal DNA restriction patterns by pulsed-field gel electrophoresis (PFGE) (15). In hospital laboratory, REA technique has been shown to be troublesome in the result interpretation (11). PFGE has been shown to be one of the most valuable tools in the epidemiological study of *P. aeruginosa*. The technique involves embedding organisms in agarose, lysing the organisms in situ, and digesting the chromosomal DNA with restriction endonucleases that cleave infrequently. Slices of agarose containing the chromosomal DNA fragments are inserted into the wells of an agarose gel, and the restriction fragments are resolved into a pattern of discrete bands in the gel by an apparatus that switches the direction of current according to a predetermined pattern. The DNA restriction patterns of the isolates are then compared with one another to determine their relatedness.

Since *P. aeruginosa* remain to be an important cause of hospital-acquired infection, especially in the ventilated patients who are particularly susceptible to colonization and infection of the respiratory tract. In order to prevent the infection with this organism, the information on the source and route of transmission would be of

great benefit for both prevention and control of this organism. By using PFGE for the typing of *P. aeruginosa* isolates from both colonized and infected patients would indicate the occurrence of the outbreak as well as the source(s) of the organism in Siriraj Hospital whether the infection come from endogenous or exogenous source and whither there are any epidemic or outbreak strains in the hospital.