

THE EPIDEMIOLOGICAL STUDY OF
***PSEUDOMONAS* spp. IN WARDS**
AT MAHARAT NAKHON RATCHASIMA HOSPITAL

Atchareeya Choungngam

A Thesis Submitted in Partial Fulfillment of the Requirements for the

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การศึกษาระบาดวิทยาของเชื้อ *PSEUDOMONAS* spp.
ในหอผู้ป่วย โรงพยาบาลมหาราชนครราชสีมา

นางอัจฉริยา ช่างงาม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ปีการศึกษา 2552

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Thesis Examining Committee

(Assoc. Prof. Dr. Griangsak Eumkeb)

Chairperson

(Assoc. Prof. Dr. Tassanee Saovana)

Member (Thesis Advisor)

(Assoc. Prof. Dr. Benjamart Chitsomboon)

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Member

(Dr. Pongrit Krubphachaya)

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(Assoc. Prof. Dr. Prapun Manyum)

Dean of Institute of Science

อัจฉริยา ช่วงงาม : การศึกษาระบาดวิทยาของเชื้อ *Pseudomonas* species ในหอผู้ป่วย
โรงพยาบาลมหาราชนครราชสีมา (THE EPIDEMIOLOGICAL STUDY OF
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การศึกษาและการวิจัยใช้แบบสอบถาม เพื่อเก็บรวบรวมข้อมูลจากหัวหน้าพยาบาล 31 คน และพยาบาลในหอผู้ป่วย 208 คน ของโรงพยาบาลมหาราชนครราชสีมา และเก็บตัวอย่างจากสิ่งแวดล้อมภายในหอผู้ป่วย 2 ครั้ง จำนวน 823 ตัวอย่าง (408 ตัวอย่างในครั้งที่ 1 และ 415 ตัวอย่างในครั้งที่ 2) เพื่อเปรียบเทียบจำนวนชนิดของเชื้อ *Ps. aeruginosa* *Ps. cepacia* *Ps. mallei* *Ps. pseudomallei* *Ps. maltophilia* *Acinetobacter baumannii* *Klebsiella pneumoniae* *Escherichia coli* และ *Enterobacter cloacae* ที่พบก่อนและหลังให้ความรู้เกี่ยวกับมาตรฐานการป้องกันการติดเชื้อ การควบคุมและป้องกันการแพร่เชื้อโรคแก่พยาบาลภายในหอผู้ป่วย นอกจากนี้ได้วิเคราะห์หาแหล่งของเชื้อโรค และวิเคราะห์สายพันธุ์ของ *Pseudomonas* ที่พบจากสิ่งแวดล้อมที่เชื่อมโยงกับการเกิดโรคในคน รวมทั้งได้เก็บรวบรวมตัวอย่างน้ำยาฆ่าเชื้อที่ใช้ตามหอผู้ป่วยจำนวน 3 ชนิดที่หอผู้ป่วยเบิกจากโรงพยาบาลมาใช้มากที่สุด เพื่อนำมาวิเคราะห์หาประสิทธิภาพในการฆ่าเชื้อแบคทีเรียของน้ำยาฆ่าเชื้อ

ผลการศึกษาพบว่า จำนวนชนิดของเชื้อโรคที่พบก่อนและหลังการให้ความรู้แก่พยาบาลภายในหอผู้ป่วยไม่แตกต่างกัน ทั้งนี้ *Pseudomonas* สายพันธุ์ที่พบในสิ่งแวดล้อม ได้แก่ *Ps. aeruginosa* และ *Ps. maltophilia* ซึ่งเป็นสายพันธุ์ที่สามารถก่อให้เกิดโรคในคนได้ และยังพบเชื้อโรคจากสิ่งแวดล้อมในหอผู้ป่วยที่ทำการเก็บตัวอย่างทุกแหล่งยกเว้นใน 70% alcohol และน้ำยาฆ่าเชื้อ providine® นอกจากนี้ผลจากการทดสอบหาประสิทธิภาพในการฆ่าเชื้อแบคทีเรียของน้ำยาฆ่าเชื้อ พบว่า sodium hypochlorite (1:20) สามารถฆ่าเชื้อ *Ps. aeruginosa* ATCC 15442 ได้ แต่ savlon® (1:100) และ pose-cresol® ไม่สามารถฆ่าเชื้อ *Ps. aeruginosa* ATCC 15442 ได้และจากการทดสอบประสิทธิภาพในการฆ่าเชื้อแบคทีเรียของน้ำยาฆ่าเชื้อที่มีประสิทธิภาพต่ำทั้ง 2 ชนิดนี้อีกครั้งทันทีหลังจากห้องยาทำการเจือจางพร้อมใช้ พบว่า savlon® (1:100) สามารถฆ่าเชื้อ *Ps. aeruginosa* ATCC 15442 ได้เพียงแคภายใน 48 ชั่วโมง ซึ่งตามกำหนดวันหมดอายุคือ 7 วัน ส่วน pose-cresol® ไม่สามารถฆ่าเชื้อ *Ps. aeruginosa* ATCC 15442 ได้ ภายใน 24 ชั่วโมง หลังจากห้องยาทำการเจือจางพร้อมใช้ทั้งที่มีกำหนดวันหมดอายุคือ 30 วัน

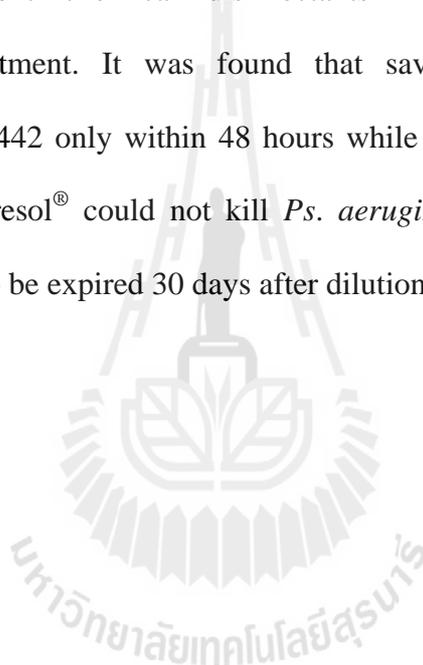
ATCHAREEYA CHOUNGNAM : THE EPIDEMIOLOGICAL STUDY
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EPIDEMIOLOGICAL STUDY/ *PSEUDOMONAS* spp./ MICROORGANISMS/
ENVIRONMENTAL SOURCES

The present study used questionnaires to collect data from 31 heads wards and 208 nurses in wards at Maharat Nakhon Ratchasima Hospital. The samples were also collected from various environmental sources within wards in 2 periods that provided total 823 samples (408 samples in period 1 and 415 samples in period 2) for comparison between number of species of *Pseudomonas* (*Ps.*) (*Ps. aeruginosa*, *Ps. cepacia*, *Ps. mallei*, *Ps. pseudomallei* and *Ps. maltophilia*), *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* found in the wards before and after giving knowledge to nurses in wards about standard precautions, control and prevention of microorganisms spreading. The environmental sources within wards of these microorganisms were also studied. *Pseudomonas* species found in the environmental sources within wards were analyzed whether they were associated with human diseases. Moreover, 3 diluted chemical disinfectants which were commonly used in ward's stock at Maharat Nakhon Ratchasima Hospital were also collected from wards to test for their bactericidal efficiency.

The results of this research showed that number of species of microorganisms were not different between before and after giving the knowledge to nurses in wards.

Pseudomonas species that were found in the selected environmental sources within wards were *Ps. aeruginosa* and *Ps. maltophilia* which were associated with human diseases. Microorganisms were found in every environmental source within wards except in 70% alcohol and providine[®] antiseptic solution. From the bactericidal efficiency test, *Ps. aeruginosa* ATCC 15442 was killed by sodium hypochlorite (1:20) but not savlon[®] (1:100) and pose-cresol[®]. The efficiency test was then repeated again for the poor efficient chemical disinfectants immediately after diluted by pharmaceutical department. It was found that savlon[®] (1:100) could kill *Ps. aeruginosa* ATCC 15442 only within 48 hours while it stated to be expired 7 days after dilution. Pose-cresol[®] could not kill *Ps. aeruginosa* ATCC 15442 within 24 hours while it stated to be expired 30 days after dilution.



School of Biology

Student's Signature _____

Academic Year 2009

Advisor's Signature _____

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CHAPTER I

INTRODUCTION

1.1 Background / Problem

Nosocomial infection refers to infection acquired in hospital by a patient who was admitted for a reason other than that infection. An infection occurs in a patient 48 hours or more after hospital admission or within 30 days after discharge. This includes infections acquired in the hospital but appearing after discharge or after admitted 48 hours (Bruce, 1987). Infections are considered nosocomial.

Nosocomial infections are significant problems throughout the world and keep increasing each year (Alvarado, 2000). For example, nosocomial infection rates range from as low as 1% in a few countries in Europe and the America to more than 40% in parts of Asia, Latin America and sub-Saharan Africa (Lynch, 1987). A prevalence survey involving 55 hospitals in 14 developing countries in four WHO regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed 8.7% of nosocomial infections (Tikhomirov, 1987).

In Thailand, the nosocomial infection surveillance in 1988 reported 11.7% of incidence rate and 7% of mortality rate or 14,000 cases. It lost about 1,000 million bahts per year (Danchaiwijitr and Choklolkaew, 1988). According to Maharat Nakhon Ratchasima Hospital reported on 4th July 2006, showed 106 cases of nosocomial infections from 1,089 admitted cases (Maharat Nakhon Ratchasima Hospital, 2006b).

The lung infections currently accounted for 15% of all hospital-acquired infections in the United States (Centers of Disease Control, 1986). Nosocomial pneumonia was associated with mortality ranging from 20 to 50% and was the most common fatal nosocomial infections.

On the basis of epidemiologic studies, it is estimated that about 15% of all hospital-associated deaths are directly related to hospital-acquired pneumonia. Any reduction in the incidence and mortality from this particular infectious complication will have a major impact upon hospital-associated mortality (Gross, Neu, Aswapokee, Van, and Aswapokee, 1980).

Pseudomonas (Ps.) aeruginosa has become a major cause of hospital-acquired infection. The treatment for *Ps. aeruginosa* infection is difficult, in part because of this organism's antibiotic resistance is common and is becoming more widespread (Wistreich and George, 1998). Ventilator-associated pneumonia research unit of Maharat Nakhon Ratchasima Hospital reported that 24 cases of *Ps. aeruginosa* were isolated from 88 infected cases and mortality rates from *Ps. aeruginosa* were 17 cases from 68 cases (Maharat Nakhon Ratchasima Hospital, 2006b) and second meticillin drug resistance was 19% in 2005 and 21% in 2006 (Maharat Nakhon Ratchasima Hospital, 2006a). Therefore, investigation of *Ps. aeruginosa* and other *Ps.* species have been done for developing the preventive measures to reduce the incidence and spread of *Ps.* species at Maharat Nakhon Ratchasima Hospital.

1.2 Research Objectives

1. To compare number of species of microorganisms cultured from the environmental sources in common wards before and after giving the knowledge to nurses in wards about standard precaution, control and prevention of microorganisms spreading at Maharat Nakhon Ratchasima Hospital.

2. To study species of *Pseudomonas* that associated with human diseases from the environment in common wards at Maharat Nakhon Ratchasima Hospital.

3. To study *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* in various environmental sources within common wards at Maharat Nakhon Ratchasima Hospital.

4. To study the bactericidal efficiency of diluted chemical disinfectants used in wards at Maharat Nakhon Ratchasima Hospital.

1.3 Scope and Limitations of the Study

1. In this study, the specimens were collected and cultured for *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* from the environmental sources in common wards at Maharat Nakhon Ratchasima Hospital. This study was divided into 2 periods: before and after gave knowledge to nurses in wards about standard precaution, control and prevention of microorganisms spreading. The comparison between number of species of *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* in these 2 periods was performed.

2. *Pseudomonas* species in this study were concentrated to common species that associated with human diseases which were *Ps. aeruginosa*, *Ps. cepacia*, *Ps. mallei*, *Ps. pseudomallei* and *Ps. maltophilia*.

3. *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* in various environmental sources within wards at Maharat Nakhon Ratchasima Hospital were also studied.

4. Diluted chemical disinfectants used in wards at Maharat Nakhon Ratchasima Hospital were studied for *Ps. aeruginosa* resistance. These disinfectants were

Savlon[®] (15% chlorhexidine gluconate: 15% cetrimide) was diluted with sterile water in a ratio 1:100.

Five gm. pose-cresol[®] was diluted with 1,000 ml. distilled water.

Sodium hypochlorite was diluted with water from faucet aerator in a ratio 1:20.

1.4 Expected Benefits

1. The finding of *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* in environmental sources within wards will promote awareness to nurses for strict precaution not to spread pathogens to patients.

2. Reduction in number of species of *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* in common wards will be found after giving knowledge to nurses in wards about standard precautions, control and prevention of microorganisms spreading.

3. The data about *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* which were yet found in environmental sources within wards after the second repeated examination will inform the head of Infection Control for control management.

4. The efficiency of the diluted chemical disinfectants used in wards at Maharat Nakhon Ratchasima Hospital should be frequently checked.



CHAPTER II

LITERATURE REVIEW

Nosocomial infections add to functional disability, emotional stress and may, in some cases, lead to disable conditions that reduce the quality of life. In addition, nosocomial infections have now become one of the leading causes of death (Ponce-de-Leon, 1991).

A characteristic of the gram negative nonfermenters, *Ps. aeruginosa* infection most often occurs in a hospital setting. Several clinical conditions are highly correlated with *Ps. aeruginosa* infection, including cystic fibrosis, burns, urinary catheters, cancer chemotherapy, or any other conditions which reduce the patient's immune responses. From any such infection, the patient may develop *Ps. aeruginosa* pneumonia or bacteremia. These conditions are serious and have mortality rates on the order of 60 to 70%. It is estimated that more than 90% of deaths in cystic fibrosis patients are due to *Ps. aeruginosa*. *Ps. aeruginosa* is a regular cause of external otitis (outer ear infection), sometimes refers to as "swimmer's ear". *Ps. aeruginosa* infection of the eye is a serious condition that may lead to perforation of the cornea with subsequent loss of the eye. *Pseudomonas* infection of the skin can occur in persons who bathe in contaminated hot tubs (Marcus, Donald, and Richard, 1997).

There are more than 300 spp. within the genus *Pseudomonas*, but of these only a few are commonly associated with human disease. *Ps. pseudomallei* and *Ps. mallei* differ from others of this genus in that they are primary pathogens and are

extremely virulent. *Ps. pseudomallei* is endemic in Southeast Asia, but infections outside of that area are uncommon. The three *Pseudomonas* most frequently seen in human disease are *Ps. cepacia*, *Ps. maltophilia* and *Ps. aeruginosa*. About two-thirds of all the clinically significant isolates of nonfermentative gram negative bacilli are *Ps. aeruginosa* (Marcus et al., 1997).

Pseudomonas is a clinically significant and opportunistic pathogen, often causing nosocomial infection. In addition to cause serious and often life-threatening diseases, these organisms exhibit innate resistance to many antibiotics and can develop new resistance after exposure to antimicrobial agents. Some *Pseudomonas* spp. that previously were considered to be the causative agents of old diseases now are being reexamined for their potential use as biological warfare agents. The current classification of the genus *Pseudomonas* is divided into 5 groups based on ribosomal RNA (rRNA)/DNA homology. More than 20 species of *Pseudomonas* that have been found from human clinical specimens, the 5 representative organisms are as follow:

Ps. aeruginosa

Ps. cepacia

Ps. mallei

Ps. pseudomallei

Ps. maltophilia

Pathophysiology

Ps. aeruginosa

Although *Ps. aeruginosa* was a common human saprophyte, it rarely causes disease in healthy persons. Most infections with this organism occur in compromised hosts. Examples of compromising conditions include disrupted physical barriers to bacterial invasion (e.g.: burn injuries, IV lines, urinary catheters, dialysis catheters, endotracheal tubes) and dysfunctional immune mechanisms, such as those occurring in neonates, cystic fibrosis (CF), AIDS, neutropenia, complement deficiency, hypogammaglobulinemia and iatrogenic immunosuppression. The pathogenesis of this organism is multifaceted and involves various toxins and proteases (e.g.: exotoxin A, lecithinase) and the glycocalyx “slime”. *Pseudomonas* infection occurs in 3 stages: (1) bacterial attachment and colonization, followed by (2) local invasion and (3) dissemination and systemic disease. In healthy children, disease is limited primarily to the first 2 stages (as in diseases such as otitis externa, urinary tract infections (UTIs), dermatitis, cellulitis, and osteomyelitis), although recent case reports describe bacteremia, sepsis, and GI infections in previously healthy children. In immunocompromised hosts, including neonates, infection can progress rapidly through the 3 stages and cause pneumonia, endocarditis, peritonitis, meningitis, ecthyma gangrenosum (EG), and overwhelming septicemia (Pollack and Young, 1979).

Ps. cepacia

In 1949, Walter Burkholder of Cornell University first described *Ps. cepacia* (now known as *Burkholderia cepacia*) as the phytopathogen responsible for the bacterial rot of onions. In the 1950s, *Ps. cepacia* was first reported as a human

pathogen that caused endocarditis. Subsequently, the organism has been found in numerous catheter-associated UTIs, wound infections, and IV catheter-associated bacteremias. In 1971, this species was reported as the causative organism of foot rot in US troops on swamp training exercises in northern Florida; it also was isolated from troops served in Vietnam's Mekong Delta. In 1972, *Ps. cepacia* was discovered as an opportunistic human pathogen in a patient with CF. Since then, *Ps. cepacia* has emerged with increasing frequency as the cause of pneumonia and septicemia in children with CF.

Ps. mallei

Ps. mallei (now known as *Burkholderia mallei*) causes glanders, a serious infectious disease of animals (primarily horses, although it also has been isolated in donkeys, mules, goats, dogs, and cats). Transmission was believed to occur through direct contact. Glanders transmission to humans was rare and presumably occurs through inoculation of broken skin or the nasal mucosa with contaminated discharges. Manifestation of the disease in humans varies, ranging from an acute localized suppurative infection, acute pulmonary infection, or acute septicemia infection to chronic suppurative infection. Fulminant disease with multiple organ system involvement occurs with septicemia infection.

Ps. pseudomallei

Ps. pseudomallei (now known as *Burkholderia pseudomallei*) causes melioidosis. (from the Greek, “resemblance to distemper of asses”) Melioidosis, also called Whitmore disease, clinically and pathologically resembles glanders but has an entirely different epidemiologic profile from *Ps. pseudomallei*. It occurs in many animals (e.g.: sheep, goats, horses, swine, cattle, dogs, cats). Transmission was

believed to occur through direct contact, although inhalation reportedly was a possible route of acquisition. Since the first description of the disease from North Queensland, Australia, in 1962, melioidosis has spread to Southeast Asia.

Ps. pseudomallei was found in contaminated water and soil. The pathogen spreads to humans and animals through direct contact with a contaminated source. In otherwise healthy hosts, disease manifestations range from acute to chronic local suppurative infections to septicemia with multiple abscesses in all organs of the body (Selina, Online, 2007).

Ps. maltophilia

Ps. maltophilia was the second most frequently isolated *Pseudomonas* spp. in clinical laboratories. In nature, *Ps. maltophilia* is found in water and in both raw and pasteurized milk. It has been associated with a variety of opportunistic infections in humans, included pneumonia, endocarditis, urinary tract infections, wound infections, septicemia, and meningitis (Barbara, Online, 2007).

The Organism Characteristics

Microbiology

Ps. aeruginosa is a motile bacillus that is usually about 2 μm . long, gram negative bacillus, oxidase positive, usually grows on Mac Conkey agar (nonfermenter). It is an obligated aerobic and grows well on most culture media. More than 90% of isolated *Ps. aeruginosa* produce a blue-green, water-soluble pigment (pyocyanin), which diffuses into the culture medium. This pigment was first observed on bandages covering infected wounds. The growing colonies give off a sweet odor variously described as grape-like or corn tortilla-like. *Ps. aeruginosa*

grows well at 42°C and produces acid from glucose (oxidative) (Marcus et al., 1997; Inglis, 1997).

Laboratory Identification

1. *Ps. aeruginosa* and the other *Pseudomonas* are flourish on ordinary nutritional media and do not ferment sugars.

2. *Ps. aeruginosa* produces a blue-green pigment as the result of the elaboration of two pigments. These aid in the identification of the organisms (James, 1998).

Habitat

Ps. aeruginosa is widely distributed in soil, water, sewage, intestinal tracts, and plants. The organism has been isolated from various materials including disinfectants, cosmetics, and foods (Wistreich and George, 1999). *Ps. aeruginosa* is an opportunistic pathogen and its presence in hospital water is a matter for concern (Graham and Edmund, 2000). Although its natural habitat is soil and water, *Ps. aeruginosa* frequently causes infections in hospitalized patients (Kathleen, 1996). *Ps. aeruginosa* is a normal inhabitant of the intestinal tract. Numerous species are present in this genus, some of which are considered as normal flora. Opportunistic infections by *Ps. aeruginosa*, *Ps. cepacia* and other *Pseudomonas* are seen in patients with burns, cystic fibrosis, intestinal surgery, or other disabilitation conditions (James, 1998).

Pathogenicity and Pathogenesis of Infections

Transmission

A variety of bacterial products, such as exotoxin elastase, proteases, endotoxic cell wall and occasionally a mucoid capsule, probably account for the

virulence associated with this organism. The organism is commonly found on plants, in areas where there is any collected water and occasionally as transient flora in the human intestine. This organism is so ubiquitous in the environment that no open wound, burn, or immune compromised patient is free from exposure (Marcus et al., 1997).

All *Pseudomonas* are resistant to the environment and survive for long periods of time in water and air, and on articles of bed clothing and the like, from which patients may become colonized (James, 1998).

Ps. aeruginosa is spreading in a number of ways, including by contamination of fingers and instruments such as urinary catheters, endoscopes, and respiratory therapy equipments. Bathing or soaking in contaminated water also can serve to transmit the organism (Wistreich and George, 1999).

Tissue invasion and damage

The ability of microorganism to invade the host is the hallmark of its virulence. This process is critical to the transformation of *Ps. aeruginosa* from a harmless saprophyte to a fully virulent pathogen. A number of factors appears to be involved in the ability of *Ps. aeruginosa* to cause invasive disease. Although the cell envelope of *Ps. aeruginosa* protects it from cellular and humoral elements of the host's immune system, its extracellular enzymes or toxins break down physical barriers to organism's penetration, further impair host defenses and render the parasite's newfound milieu more conducive to its physical, nutritional and reproductive requirements (Pollack, 1984).

Virulence factors

Ps. aeruginosa produces an A-B toxin with exactly the same mode of action as the diphtherial toxin. This toxin is known as exotoxin A and is able to prevent protein synthesis by infecting host cells.

1. Exotoxin S is also an ADP - ribosyl transferase that acts on one of the G proteins. This organism requires exotoxin S in order to be virulent, but the mode of action of the toxin is unknown.

2. Phospholipases, proteases, a cytotoxin, and an iron-binding siderophore contribute to the virulence of *Ps. aeruginosa* (James, 1998).

Exotoxin A (ETA)

Liu (1974); Liu, Yoshii, and Hsieh (1973) remark that purified lethal toxin from *Ps. aeruginosa* is called exotoxin A (ETA). It is produced by approximately 90% of clinical isolation (Bjorn, Vasil, Sadoff, and Iglewski, 1977; Pollack, Taylor, and Callahan, 1977). This single polypeptide toxin composes of acidic amino acids, has an isoelectric point of 5.0-5.1 and has molecular weight in the range of 50,000 - 71,500 (Rose, Heckman, and Unger 1973; Kessner and Lepper, 1967). ETA conforms to the A-B structure functional model like many bacterial toxins in that one portion of the molecule (fragment B) is necessary for interaction with the eukaryotic cell receptor, while the other, (fragment A) is catalytic portion (Vasil, Chamberlain, and Grant, 1986). This toxin is heat labile, being readily destroyed by heating at 60°C for 15 minutes (Morrison and Wenzel, 1984; Liu, 1974). ETA can be converted to toxoid by formalin treatment (Dogget and Robert, 1979). Its production is regulated by environmental iron concentration. If iron levels are sufficient for optimum growth, the synthesis of toxin is repressed (Reynolds, 1985). The mean lethal dose (LD50)

was 60-80 ng for 20 gm. mouse (Callahan, 1976). Subsequent studies suggested that ETA inhibited protein synthesis. The mechanism of action was presented elegantly by Iglewski and Kabat in 1975 who reported that ETA catalyzed the transfer of the ADP-ribosyl moiety of NAD onto eucaryotic Elongation factor 2 (EF₂) like diphtheria toxin. This process inactivates EF₂ and halts protein synthesis especially in the liver (Iglewski and Kabat, 1975). Mechanism by which *Ps. aeruginosa* ETA and diphtheria toxin affect cells, ETA is 10,000 times more lethal for experimental animals than *Pseudomonas* lipopolysaccharide (Pollack, 1980; Pollack and Young, 1979). Intravenous injection causing severe hypotension and death, hepatotoxicity and lung haemorrhage are also found (Atik, Liu, Hanson, Amini, and Rosenberg, 1968; Bergan, 1981).

Proteases

Ps. aeruginosa isolated from clinical specimens produce several proteases, elastase and alkaline protease, contributed to adhere and caused skin haemorrhage and necrosis of connective tissues (Ohman, 1980). The corneal damage produced by purified proteases has been observed in experimental animals. Proteases of *Ps. aeruginosa* are also capable of inducing alveolar necrosis and haemorrhage in the lungs of experimental animals similar to that observed in patients with pneumonia caused by *Ps. aeruginosa* (Gray and Kreger, 1979). It has been suggested that elastase is responsible for dissolution of the elastic lamina of blood vessels, which is an important pathologic characteristic of these lesions (Mull and Callahan, 1965). It can also inactivate complement components and cleave immunoglobulins (IgG and IgA) (Schultz and Miller, 1974). Elastase and alkaline protease were also reported to

cause proteolytic inactivation of human interferon (IFN- γ), interleukin-2 and tumor necrosis factor (TNF) (Parmely, Gale, and Clabaugh, 1990).

Phospholipase C and cytotoxin

These enzymes are able to degrade phospholipids and lecithin, hemolyze erythrocytes and cause damage of most eukaryotic cells including polymorphonuclear leukocytes (Bone, 1993).

Antibiotic resistance

Ps. aeruginosa is well known for its intrinsic resistance to many commonly used antibiotics. Previously the susceptibility of this organism to the advanced generation cephalosporins, ureidopenicilins, aminoglycosides, quinolones and carbapenems were acceptably good and this provide clinicians with wide varieties of antibiotics to choose for treating patients with *Ps. aeruginosa* infections. Unfortunately now, this organism is frequently associated with multiple resistance acquired during therapy and presents as therapeutic difficulties in a number of serious *Pseudomonas* infections (Watanabe, Iyobe, Inoue, and Mitsuhashi, 1991; Traub, Scheidhauer, Leonhard, and Bauer 1998; Kinoshita, Sawabe, and Okamura; 1997; Jones, Pfaller, Marshall, Hollis, and Wike, 1997). For example in the early 1990s, 11.8% of the *Ps. aeruginosa* strains responsible for nosocomial infections in the United States were found to be resistant to imipenem, the powerful antibiotic frequently used for treating complicated gram negative sepsis. The distribution of multiresistant strains and the pattern of resistance of these strains to various antibiotics were studied. The reason for this change in susceptibility trends is still unclear. However this may partly be due to the inappropriate use or prolonged exposure of patients to the broad spectrum antibiotics (Troillet, Samore, and Carmeli,

1997). The mechanisms of resistance to each class of antibiotics are diverse. The outer membrane of *Ps. aeruginosa* is intrinsically about 10 times less permeable than *Escherichia coli*, accounting for the fact that many antibiotics active against *Ps. aeruginosa* are not effective. This has been known to be due to the presence of MexAB-OprM protein which resulted in reduced permeability and efflux of many types of antibiotics. Three genetically distinct systems which are involved in reduced permeability and efflux have been described, each composed of 3 proteins. A cytoplasmic membrane protein (Mex B, Mex D or Mex F) is thought to act as an energy dependent pump with broad substrate specificity. A second protein (OprM, OprJ, OprN) is located in the outer membrane, acting like a porin. A third protein (MexA, MexC or MexE) is located in the periplasmic space and thought to be the link protein (Michea-Hamzhepour, Lucain, and Pechere, 1991). Overproduction of the MexAB-OprM protein system via chromosomal mutation (*nalB*) increases the resistance of *Ps. aeruginosa* to quinolones and β -lactams (except imipenem) (Gotoh, Itoh, Tsujimoto, and Yamagishi, 1994). The chromosomal mutations *nfxB* and *nfxC* which release, repress production of two other systems, MexCD-OprJ and MexEF-OprN respectively, endow the organism resistant to many other antibiotics including quinolones (Gotoh, 1998). No other bacteria so far have been shown to possess such an efficient network of efflux. In addition to permeability and efflux, *Ps. aeruginosa* is remarkable for its capacity to accept foreign genetic materials such as plasmids and transposons, which brings additional mechanism of resistance. A single or an interplay of few mechanisms of resistance may be present in a single strain of *Ps. aeruginosa*. For example aminoglycoside resistance may be due either to aminoglycoside inactivating enzymes or to diminished cell permeability. Resistance

to advanced generation beta-lactams can be conferred by plasmid or chromosomal mediated β -lactams enzymes, diminished permeability through outer membrane channels as well as alteration in penicillin binding protein (PBP). *Ps. aeruginosa* may become resistant to imipenem via two mechanisms:

1. Concurrent effects of a chromosomal β -lactams activity and decrease permeability due to the loss of a specific outer-membrane protein or porin (OprD2).
2. Plasmid mediated metallo β -lactams capable of hydrolyzing carbapenems efficiently (Senda et al., 1996).

The former mechanism is more common. The latter has only been reported in Japan so far but has a potential of spreading rapidly since it is plasmid mediated. Troillet N. and co-workers had reported that treatment with imipenem was a major risk factor for the clinical detection of multiresistant strains *Ps. aeruginosa* (Troillet et al., 1997). Resistance to fluoroquinolones appears to be due primarily to alterations in outer membrane proteins that influence antibiotic entry and to a lesser extent to alterations in target DNA gyrase (Daikos, Lolans, and Jackson, 1988).

Ps. aeruginosa has intrinsic resistance to most available antibiotics, leaving aminoglycosides, anti *Pseudomonas* penicillins, newer cephalosporins, imipenem and fluoroquinolones as treatment options for systemic infection. Some institutions have found that despite isolating patients with antibiotic-resistant *Ps. aeruginosa*, the incidence of colonization with these strains has continued to increase, in part paralleling the increasing use of aminoglycoside antibiotics (Turano and Peretti, 1977). Most strains of *Ps. aeruginosa* are resistant to relatively high levels of most antibiotics in use, for two reasons. First, entry of antibiotics into the periplasmic space and further into the cytoplasm is considerably more restricted than in other gram

negative bacteria, because the porins of the outer membrane, under the influence of divalent cations, limit the passage of water-soluble molecules. Second, high-level resistance mechanisms are present and include production of several beta-lactamases and aminoglycoside-inactivating enzymes, acetylation of chloramphenicol and efficient expulsion of tetracycline. Both plasmid and chromosomal genes are involved. The plasmids are often transmissible, not only within the genus, but to other negative pathogens as well (Lory, 1990). Acquired additional resistance involving modifying enzymes is particularly associated with topical antibiotic use and with sites, e.g. bladder, where high levels of antibiotic are achieved. An additional form of acquired resistance does not involve modifying enzymes but is apparently the result of reduced permeability associated with a change in outer membrane proteins (Govan, 1998).

Nosocomial Infection caused by *Ps. aeruginosa*

Search was sometime required for *Ps. aeruginosa* in the investigation of consumer's complaints and problems with poor quality in distributed water. It is probably derived from human or animal faeces, but it is not universal in faeces. It is able to grow in water containing nutrients and particularly in those parts of a water distribution system which have low flows and are warm. *Ps. aeruginosa* is an opportunistic pathogen and its presence in hospital water is a matter for concern (Matthew, 1990).

Epidemiology

Ps. aeruginosa is primarily a nosocomial pathogen and frequently causes disease (Centers for Disease Control, 1983). The National Nosocomial Infection Surveillance system in the United States has characterized the frequency of non

fermentative aerobic gram negative bacilli every year. About 10% of the reported nosocomial infections were caused by *Ps. aeruginosa*. *Ps. aeruginosa* accounted for 11% of all the urinary tract nosocomial infections making it to be the second most common pathogen. It is the leading cause of hospital acquired pneumonia (accounted for 20% of all isolated organisms) (Arnow and Flaherty, 1996).

Extensive culture studies to identify inmate reservoirs for *Ps. aeruginosa* in hospitals have been done extensively since the 1950s. This organism can actually be cultured from practically any moist area and from fluids including disinfectants, equipments and surfaces (Stephenson, Heard, Richards, and Tabagchali, 1985; Earnshaw, Clark, and Thom, 1985; Correa, Tibana, and Gonitijo-Filho, 1991). Some of these reservoirs have been proven to be the source of point source outbreaks in several hospitals (Graham, 2000). Patients in special care units are often colonized by *Ps. aeruginosa*. This is an important source for propagation of infections among these patients. These patients are exposed to broad spectrum antibiotics, medical devices and hands of health care workers, therefore they may acquire the organism and harbour the organism for a significant period of time (Murthy, Baltch, and Smith, 1989). Colonization rates of patients in the intensive care units, oncology units, surgical units and the neonatal intensive care units ranged between 13-39%, 4-58%, 19-43% and 2-51% respectively (Murthy et al., 1989; Grundmann, Kropec, Hartung, Berner, and Daschner, 1993).

Ps. aeruginosa is cosmopolitan in its distribution. It is isolated from soil, water, plants, and animals, including man. It is occasionally pathogenic for plants as well as animals. The epidemiology of *Ps. aeruginosa* reflects its predilection for a moist environment. This is apparent in its natural habitat, where its associations with

soil and water are closely related and its identification on plants is a function of humidity. Moisture is also a critical factor in hospital reservoirs of *Ps. aeruginosa* such as respiratory equipment, clean solutions, medicines, disinfectants, sinks, mops, food mixers, vegetables and so on. Human *Pseudomonas* disease is also associated with water-related reservoirs outside the hospitals (Matthew, 1990).

Potential reservoirs such as uncooked vegetables, hospital sinks, or even flowers in patient's rooms are suspected sources of endemic *Ps. aeruginosa* strains. Discrete hospital-acquired outbreaks (epidemics) have been more definitively traced to specific reservoirs such as respiratory equipments, endoscopes, transvenous pacemakers, contaminated antistatic mattresses, antiseptics, orthopedic plaster, operating room suction apparatus, contaminated nursery formula, physiotherapy pool, and so on. Patient to patient transmission of *Pseudomonas* via the hands of hospital staffs or by other fomites is often assumed but difficult to prove (Matthew, 1990).

The pathogenesis of *Ps. aeruginosa* infections must be understood in the context of its being an opportunistic pathogen. It rarely causes disease in healthy persons, although it is a common human saprophyte. Its adaptability to a wide variety of physical conditions, minimal nutritional requirements, and relative resistance to antibiotics allow it to survive in large numbers in close proximity to its prospective host (Matthew, 1990).

Disease

1. Burn patients are often infected because the loss of skin expose their open flesh to ready colonization of this moist, nutrient-rich surface.
2. This bacillus is a major cause of death in patients with cystic fibrosis who invariably become colonized at some time in their lives.

3. Postsurgical intestinal or urinary tract infections with *Ps. aeruginosa* infections are a third category of disease commonly seen in patients (James, 1998).

Clinical Manifestations

Bacteraemia

Nosocomial blood stream infections (bacteraemia) occur at a rate of 1.3 to 14.5 per 1,000 hospital admissions and are believed to lead directly to 62 per 5,000 deaths per year in the United States (Pittet and Wenzel, 1995). Incidence varies with the type of patient population, the size of institution, the length of hospital stay and the ward location within an institution (Gatell, Trilla, and Latorre, 1988).

Ps. aeruginosa bacteraemia is associated with significant morbidity and mortality especially in the immunocompromised and critically ill patients. Factors predispose patients to *Ps. aeruginosa* infections are cancer, immunoglobulin deficiencies, diabetes mellitus, burns, prematurity and other immunocompromised states. Overall incidence of *Ps. aeruginosa* bacteraemia ranged between 1-1.8 per 1,000 admissions (Sherertz, 1983; Bisbe, Gatell, and Puig, 1988). However the incidence rates among patients with burn injuries and patients with cancer may be as high as 5 per 1,000 admissions (Bodey, Jedeja, and Elting, 1985; Hsueh, Teng, and Yang, 1998). Infections usually occur within the first two weeks of chemotherapy in cancer patients due to rapid decline of neutrophil counts. In a large study evaluating 410 episodes of *Ps. aeruginosa* bacteraemia, shock occurred in 33% of the patients studied (Bodey et al., 1985). An interesting phenomenon has been observed in many institutions treating cancer patients. A decline number in the overall incidence of *Ps. aeruginosa* bacteraemia has been noted during the mid 1980s till early 1990s. However for the past few years, the trend seemed to reverse and was a cause for

concern since the concurrent isolation of several multiresistant strains among the isolates caused case clusters (Aquino and Pappo, 1995).

Bacteraemia can be primary or secondary infections. Cases of primary *Ps. aeruginosa* bacteraemia were often related to the use of intravenous devices (20%) and infusion of contaminated fluid (Velasco et al., 1997; Leigh et al., 1995). Occasionally primary bacteraemia may also arise following endoscopic procedures especially in the presence of underlying mucosal lesions or immunocompromised states (Earnshaw, Clark, and Thom, 1985). Studies in animal models have demonstrated that translocation of bacteria through intact mucosa can occur if normal balance of microbial flora is altered (Deitch, Winterton, and Berg, 1987). This has also been observed in patients who have been exposed to broad spectrum antibiotics and those with gut colonization by this organism where no appearance of primary source was detected. *Ps. aeruginosa* may enter the bloodstream through invasion from a primary site. Virulence factors such as exotoxin A and other extracellular enzymes as well as the underlying conditions of the hosts are factors contributing to dissemination of the organisms (Arnou and Flaherty, 1996).

Ps. aeruginosa bacteraemia is clinically indistinguishable from any other gram negative sepsis. Fever, tachycardia and hypotension are common findings. Small round indurated skin lesions termed “ecthyma gangrenosum” may be pathognomonic but rarely observed (Bodey et al., 1985; Dorff et al., 1971).

Mortality rates associated with this infection have been in the range up to 70 to 90% before 1970. The rates have declined to 40-50% over the past few decades. This has been associated with the administrations of effective anti *Pseudomonas* antibiotics empirically in patients presumed to have gram negative sepsis (Fergie et

al., 1994). With the isolation of several multiresistant strains now, upclinicians may be facing therapeutic difficulties in the near future unless some form of effective control is carried out. Therapeutic failure rates of 70% have been observed in centres with persistence of the multiresistant strains of *Ps. aeruginosa* isolates.

Urinary tract infection

Urinary tract infection (UTI) is the most common type of nosocomial infection in both acute care hospitals and long-term care facilities. It accounts about 15% of nosocomial infection in Auckland hospital (Nicholls and Morris, 1997).

Ps. aeruginosa accounts for about 12% of nosocomial urinary tract infections and ranks the third in frequency at this site behind *Escherichia coli* and *Enterococcus* (Jarwis and Martone, 1992). Incidence rate of *Ps. aeruginosa* urinary tract infection was 3.1% in endemic setting and 4.5% in an epidemic setting (Krieger, Kaiser, and Wenzel, 1983).

Adherence usually precedes most invasive UTI. *Ps. aeruginosa* adheres well to uroepithelial cells. Colonization of the rectum perineum or urethra predisposes to infection (Bultitude and Eykyn, 1973). Urine is an excellent growth medium for common urinary tract pathogens (Chambers and Kunin, 1987). Indwelling catheter disallowed complete cyclic emptying of the bladder and creates a small pool of residual urine in which microorganisms can multiply, thus bringing about infections. Urinary catheterization has reduced infections due to *Escherichia coli* but has definitely increased the risk of *Ps. aeruginosa* UTIs. Over one-third of chronically catheterized patients have evidence of acute renal inflammation at autopsy (Warren, Muncie, and Hall-Craggs, 1988).

Ps. aeruginosa bacteraemia in catheterized patients often resolved spontaneously within 2-3 months (Warren, Tenney, Hoopes, Muncie, and Anthony, 1982). Necrotizing infection of the bladder or kidney is extremely rare and pyelonephritis as a complication of bacteriuria is uncommon (Arnow and Flaherty, 1996). Nevertheless, on occasion, the infection can be quite severe and cause a hemorrhagic cystitis or secondary bacteraemia. *Ps. aeruginosa* bacteraemia following urologic procedures has been reported (Garibaldi, 1993).

Infections of burn wounds

Patients suffering from burn injuries are at high risk of getting nosocomial infections. The lost skin integument which is the primary barrier to organism invasion, together with present of serum proteins and the necrotic tissue in the burn eschar promotes multiplication of microorganisms to high concentrations. Microorganisms that normally reside on the skin surface can grow in the presence of these factors and lead to an invasive burn infection (Ang and Lee, 1997). Thermal injury also depresses both local and systemic immunity (Heideman and Bengtsson, 1992).

Ps. aeruginosa is one of the most common burn wound pathogen and it has colonized or infected more than one fourth of patients in several series (Pruitt, 1974). Recent study however showed that *Ps. aeruginosa* burn wound infections were becoming less common (Ang and Lee, 1997).

There is evidence that *Ps. aeruginosa* burn wound infections may arise from contamination from faeces. Wounds around the buttock, perineum, lower abdomen and the inside of upper thighs were most often infected. Hydrotherapy equipment, sink faucets, faucets handles, bars of soap and towel rack were also known to be

potential contaminated sites and have been reported as source of wound infections (Shulman, Terry, and Hough, 1971).

Development of antibiotics resistance which is related to extensive use of antibiotics has also been reported (Mayhall, 1993). When *Ps. aeruginosa* colonizing burn wounds become resistant to topical antimicrobial agent used, the risk of uncontrolled growth and invasion of variable tissues are more likely to occur (Mayhall, 1996). Silver-resistant *Ps. aeruginosa* have been reported in burn unit (Bridges, Kidson, Lowbury, and Wilkins, 1979). Manson and associates observed the significant positive association between length of stay and colonization with *Ps. aeruginosa* (Manson et al., 1992).

Invasion of the burn by *Ps. aeruginosa* may occur either abruptly or slowly. In a typical case, the burn wound developed heavy, green pigmented and foul-smelling discharge. In rapidly advancing and invasive infections, the eschar may develop to shaggy green exudate and later progress to form patchy, black areas of necrosis. Bacteraemia and septicemia are common complications of extensive burn wound infections caused by *Ps. aeruginosa*. Patients usually become hypothermic and have a depressed white blood cell count, clinical ileus and mental confusion are suggestive of severe and overwhelming sepsis. Ecthyma gangrenosum is often seen before death in patients with septicemia (Tompkins and Burke, 1992).

Respiratory infections

Most bacterial nosocomial pneumonias occur by aspiration of bacteria colonizing the oropharynx or upper gastrointestinal tract of the patients. Intubation and mechanical in ventilation greatly increase the risk of bacterial nosocomial pneumonia because they alter first-line patient's defenses (Chervret, Hemmer, Carlet,

and Langer, 1993; Schaberg, Culver, and Gaynes, 1991; Bartlett, Keefe, Tally, Louie, and Gorbach, 1986).

Lower respiratory tract infections with *Ps. aeruginosa* occur almost exclusively in persons with compromised local respiratory or systemic host defense mechanisms. Primary pneumonia occurs in patients with chronic lung disease, congestive heart failure, or both. Exposure to the hospital environment, particularly in an intensive care setting, use for respiratory inhalation equipment, and prior antibiotic therapy increase the likelihood of such infection (Matthew, 1990).

Pneumonia

Data from the Center of Disease Control National Nosocomial Infection Study (NNIS) indicates that lower respiratory tract infection is second leading site of hospital acquired infections (Horan, Culver, and Jarvis, 1988) but be the leading cause of death (American Thoracic Society, 1995). Nosocomial pneumonia accounts for about 13% of all nosocomial infections in United States (Horan et al., 1988). The incidence was higher ten to twenty folds in ICU patients. The incidence among intubated patients was seven to twenty one times higher than non intubated patients (Celis et al., 1988). The mortality rates for ICU patients with pneumonia were two to ten times higher and further increased in patients with pneumonia caused by *Ps. aeruginosa* (Leu, Kaiser, Mori, Woolson, and Wenzel, 1989). Bilateral infiltrates on chest x-ray and respirator failure were independent risk factors for mortality (Celis et al., 1988).

Nosocomial pneumonia may be divided into early onset and late onset. The latter is where *Ps. aeruginosa* and other gram negative bacilli are commonly isolated. They are acquire from the hospital and often exhibit high level of resistance to

antibiotics (Craven, Steger, and Barber, 1993). It is more often due to direct contamination of trachea with organisms from environments or patient reservoirs (Schwartz et al., 1978).

In mechanically ventilated patient, the leakage of bacteria around the cuff of the endotracheal tube lead to colonization which is the initial step in progression to pneumonia (Leu et al., 1989). *Ps. aeruginosa* infection can lead to a necrotizing bronchopneumoniae in the case of lung infection (Craven et al., 1993). *Ps. aeruginosa* is the most important pathogen causing ventilator associated pneumonia (Rello and Torres, 1996), more importantly still, *Ps. aeruginosa* is the leading cause of death among intubed patients with pneumonia (Rello et al., 1993).

Specific problems in the treatment of *Ps. aeruginosa* pneumonia are the recurrence event after only several weeks (Silver, Cohen, and Weinberg, 1992) and emergence of resistance to the antibiotics used for treatment (Fink et al., 1994). Even with potent antibiotics such as ciprofloxacin or imipenem, the prognosis remains very poor when *Ps. aeruginosa* is isolated from blood or respiratory tract cultures in severe pneumonia cases (Fink, Snyderman, and Neiderman, 1994).

Control and Prevention

Pseudomonas infections rely on proper aseptic techniques when dealing with burns and open wounds. *Pseudomonas* spp. are able to grow in water with minimal nutrients and are found in such places as water baths used for heating baby bottles, hot tubs, vases for fresh-cut flowers, and water pumps in humidifiers. Certain precautions, such as not allowing fresh-cut flowers in critical areas of a hospital and routine monitoring of water held in baths and air systems, help reduce the hazard of these

infections. *Pseudomonas* spp. are among the most resistant vegetative bacterial cells to chemical disinfectants (Marcus et al., 1997).

Hand washing

There are two types of hand washing:

1. Social hand washing. This should be carried out:

routinely before and after coming into contact with patients.

when starting work.

when going off duty.

when they become visibly dirty.

when they are contaminated with body fluids or organic matter.

after visiting the toilet.

after removing gloves.

after a non sterile procedure.

contact with patients during ward rounds or routine procedures such as bed making or lifting should be followed by decontamination of the hands with alcohol chlorhexidine or a soap and water hand-wash.

2. Aseptic hand-washing. This type of hand washing should be used when aseptic procedure is about to be performed on a patient (e.g. introducing central venous pressure lines, peripheral cannulae or urinary catheters). It is a shorter version of the surgical hand wash and requires meticulous cleaning of the hands and the use of a sustained action disinfectant. It is usually accompanied by the wearing of gloves (Shaheen, 1992).

Procedure for hand-washing

Remove all rings, jewelry (including watch) and roll up the sleeves.

Wet the hands under running water and apply a recommended amount of the hand-wash provided to the palms of the hands.

Rub to make a lather.

Rub the hands together and then cup them around each other to massage all the finger tips properly, massaging the thumbs and the webs of the fingers.

Wash the wrists and backs of the hands.

Rinse the hand thoroughly under running water.

Dry thoroughly with several pieces of paper towels or single-use cotton towels.

If washing for an aseptic procedure.

Do not touch any non-sterile surface.

Wear gloves.

Remove gloves after the procedure, wash hands and dry thoroughly (Shaheen Mehter, 1992)

Hand disinfection

Sustained-action disinfectants with alcohol (rub) should be used.

When moving from one patient to another.

After non-sterile duties not involving body fluids.

After handling or touching a potentially contaminated surface.

Before touching a neutropenic or high-dependence patient.

All hand disinfection agents should be kept in the sterile dispensers that deliver a known quantity of soap or disinfectant. The container and nozzle must be cleaned regularly to prevent contamination and blocking. Open containers of disinfectant and soap should not be left on ward wash-hand basins as they can become contaminated with bacteria. When empty, the disinfectant containers should be returned to the Pharmacy or Domestic Department to be washed, cleaned and refilled. Defective pumps must be replaced immediately (Shaheen, 1992).

Soap and water

Soap and water remove most organic contamination and are acceptable as a social-hand wash. However, bars of soap may be left lying in pools of water, where they become contaminated with multiply antibiotic-resistant gram negative bacilli, which are then transferred to the hands of staffs and then to patients. If bar soaps are used they should be stored dry—either on a piece of string or fixed to the wall by magner holders. Medicated soap, which incorporates a bactericidal agent (e.g. triclosan or irgasan-Cidal soap) is useful in reducing the transmission of methicillin-resistant *Staphylococcus aureus*. Soap and water should be supplemented with an alcohol-containing sustained action disinfectant prior to carrying out for an aseptic technique (Shaheen, 1992).

Soap dishes and dispensers

Soap dishes are rarely necessary, and may encourage bacterial growth. If used, they should be washed and dried daily. The nozzles of liquid soap dispensers should be cleaned daily to remove residues, and the outside should be cleaned and dried. Disposable cartridge-type refills with an integral nozzle are preferred, but they tend to be expensive. If non-disposable reservoirs are used, topping up should be

avoided and the inside of containers should be cleaned and dried before refilling. In cartridge-type dispensers, the channel and reservoir between the refill and nozzle, if not disposable, require periodic cleaning. Liquid soaps used in hospitals should contain a preservative (e.g. 0.3% chlorocresol), which should prevent bacterial growth during periods of use (Graham, 2000).

Hand-wash basins

These should be available in all wards, treatment rooms, saline rooms and isolation cubicles.

A clinical hand-wash basin has

Elbow-operated mixer taps.

A deep bowl to avoid splashing and contamination.

No overflow.

No recesses where water may collect.

No drain-hole plug, so that water cannot be held in the basin.

Hand operated single taps should be turned off with the paper-or cloth towel that was used to dry the hands. Wash-hand basins should be used for hand-washing only and should have a clearly displayed sign: CAUTION-HOT WATER and HAND-WASH ONLY. The two-bowl system with a communal towel is not recommended, it is a source of cross-infection (Shaheen, 1992).

Siting the wash basins

Wash basins should be in the easy sight and reach of all hospital staffs and visitors (no-one will use a wash basin they cannot see).

Basins should have adequate, wall-mounted hand disinfectants.

Drying facilities should be close by.

In some countries, the wash-hand basin on the ward is used as a kitchen sink by the patient's relatives. This has disadvantages:

The staff cannot use the wash basin for hand disinfection.

The sink may be blocked with food debris and over-flow.

Vermin and pests may be attracted to the ward.

It is extremely unsightly.

Basins must be provided to dispose of waste for relatives and visitors use in the kitchen, or sluice, and relatives should be educated about hand hygiene (Shaheen, 1992).

Hand drying

Drying is an essential part of hand disinfection. Wet hands have higher bacterial counts and permanently wet hands become chapped and dry (Shaheen, 1992).

Paper towels

These are most often used to dry the hands. However, the quality is usually poor and several sheets are needed to dry the hands properly. A good absorbent quality is recommended (Shaheen, 1992).

Cotton towels

These are perfectly acceptable for social hand-washing provided that they are on a roller and are laundered regularly. A small face-towel for single use (be laundered before re-use) is also acceptable. Common-use cotton towels that are left lying next to a sink are dangerous and can result in cross-infection with gram positive cocci and gram negative bacilli (Shaheen, 1992).

Bedding

Bedding can rapidly become heavily contaminated with colonized skin scales. Frequent changing is therefore of limited value in controlling the spread of infection.

Cotton blankets should be used, and these should be changed on discharge of the patient or if they become soiled or contaminated with potentially infectious spillage.

Sheets should be changed on discharge of the patient and also at least twice weekly and if soiled, wrinkled, stained or contaminated with potentially infectious materials (Graham, 2000).

Prevention and treatment

1. It is very difficult to prevent colonization of burn patients and of those with cystic fibrosis because of the ubiquitous distribution and resistance of the *Pseudomonas*.

2. The *Pseudomonas* are typically resistant to several antibiotics. The quinolones and third generation beta-lactams are often recommended (James, 1998). *Ps. aeruginosa* infections are very resistant to therapy. The remarkable metabolic capabilities and diverse plasmids associated with these organisms have resulted in the development of a high level of resistance to a broad range of antibiotics. A number of new antibiotics have been specifically designed for treatment of *Pseudomonas* infections, but as yet, the ideal anti *Pseudomonas* compound has not been discovered (Marcus et al., 1997).

The study of Rello et al., 1993 described 113 mechanically ventilated patients judged to have Ventilator associated pneumonia (VAP) on the basis of clinical criteria. In 100 (88.5%) of these patients, a causative agent for VAP was

identified by evaluating cultures from either blood, pleural fluid, or lower airway secretions obtained bronchoscopically (Broncho Alveolar Lavage or Protected Specimen Brush). Antibiotic therapy was changed on the basis of the microbiologically positive culture result in 51 patients (51%). Among these patients, 27 (52.9%) were seemed to have received inadequate antibiotic therapy based on the pathogens isolated. The most common reason for inadequate initial empiric antibiotic therapy was the isolation of *Ps. aeruginosa* resistant to at least 1 of the prescribed antibiotics; this was found in 20 cases (74.1%). The crude mortality rate among the patients who received inadequate antibiotic therapy was found to be significantly greater than the mortality rate among patients who received inadequate initial empiric antibiotics (63% and 41.6%, respectively; $p = 0.06$). More important, the mortality attributed to VAP was also significantly greater among the patients who received inadequate initial therapy than among those who were treated adequately (37% and 15.6%, respectively; $p < 0.05$).

Research papers

Luckana Chirtreecheur (1986) studied on the incidence rate of respirator associated pneumonia and related factors in 3 surgical intensive care units of Siriraj Hospital. Fifty patients who were on respirators were studied while another group of fifty patients who were intubated or tracheostomized but not on respirators served as a control group. The incidence rate of pneumonia in patients on respirators was higher than that in the control group but not statistical significant. The causative bacteria of the pneumonia in this study were *Ps. aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter anitratus*, *Proteus* spp. and *Staphylococcus aureus*.

Salisa Wisutthirate (2004) studied a cross-sectional analysis of 305 intubated patients admitted in Trat Hospital in order to determine the incidence of nosocomial pneumonia (NP) and risk factors. Studied patients were interviewed by using questionnaires and case records were collected. Secretion specimens from patients with nosocomial pneumonia were collected for bacterial cultures. The result of secretion cultures from 117 patients with nosocomial pneumonia showed that 64.96% were negative and 35.04% were positive. The microorganisms presented in the cultures were *Klebsiella pneumoniae* and *Klebsiella* spp. 32%, *Acinetobacter anitratus* and *Acinetobacter* spp. 24%, *Enterobacter* spp. 10%, *Ps. aeruginosa* and *Ps.* species 8%, *Staphylococcus aureus* 8%, *Staphylococcus epidemidis* 10%, and Methicillin resistant *Staphylococcus aureus* 6% respectively. There were 36 patients infected with one type of bacterial microorganisms and 5 patients infected with multiple bacterial microorganisms.

Wasinee Thanagrumetha (1991) studied the epidemiology of *Ps. aeruginosa* infection in 6 wards of Siriraj Hospital; 3 intensive care wards and 3 general wards. The percentage of cross infection (24-86%) in each ward was significantly different ($p < 0.05$). Cross infection had occurred more frequently in intensive care ward (86%) in traumatic surgical ward no.4, whereas lower cases were found in general ward (24%) in pediatric ward no.2. *Ps. aeruginosa* cross infection in each ward was usually resistant to antibiotics.

Yupawadee Rutanawaropas (1995) studied a total of 400 isolates of *Ps. aeruginosa* dividing into 2 main groups by sources of specimens. Two hundreds *Ps. aeruginosa* isolated from clinical specimens of patients attending in 8 hospitals and other 200 *Ps. aeruginosa* which isolated from 320 wastewater samples from those

eight hospitals and from other 8 communities during 10 weeks of July to September 1993. High frequencies of *Ps. aeruginosa* isolation were found in wastewater samples from both the hospitals and environments, with or without treatment before disposal. It was detected in 215 samples (67.2%), mostly from untreated wastewater, and was not detected in 105 samples (32.8%), mostly from treated wastewater. There was a progressive reduction in the *Ps. aeruginosa* count per milliliter from only 3 in 4 of treated hospital wastewater. This studied suggested that *Ps. aeruginosa* might cause further health problem because the majority of these isolates were highly resistant to many antibiotics that might transfer R-factor to other bacteria in natural water and be back to cause infection in hospitalized patients who were prone to infection with severe outcome and in any populations with health risks. Thus this study emphasize the significance of hospital wastewater without effective treatment as a reservoir and a source of distribution of large numbers of *Ps. aeruginosa* with highly resistant to multiple drugs into a water environment and may further become a serious threat to public health in future.

CHAPTER III

RESEARCH METHODOLOGY

3.1 Methodology

This study was performed by survey and experiments using the questionnaires as the data collection tools from 31 head wards and 208 nurses in wards at Maharat Nakhon Ratchasima Hospital. Furthermore, the cultures were collected from the environmental sources and analyzed for number of microorganisms which were commonly found in the wards. Specimens from various environmental sources within every ward were taken and cultured in 2 periods: before and after giving knowledges to nurses about standard precautions, control and prevention of microorganisms spreading. The laboratory results and the bacterial sources from the first period were reported to the nurses and the interval of these 2 periods was 2 months.

In addition, the bactericidal efficiency of stock diluted chemical disinfectants used in wards at Maharat Nakhon Ratchasima Hospital was also studied.

3.2 Population and Sampling

The study population consisted of 31 head wards and 450 nurses from 31 wards at Maharat Nakhon Ratchasima Hospital.

In period 1 and 2, samples from various environmental sources were taken from 31 wards of 6 departments at Maharat Nakhon Ratchasima Hospital which were

8 wards of Surgery, 8 wards of Medicine, 6 wards of Pediatrics, 5 wards of Orthopedics, 3 wards of Obstetric and Gynecology and 1 ward of Eye and Ear Nose Throat.

Questionnaires 1 were distributed to the head wards in 31 wards at Maharat Nakhon Ratchasima Hospital.

Questionnaires 2 were distributed to the nurses in wards. The number of Questionnaire's answers was fixed by Krejcie and Morgan (Yut, 2002) that must be at least 208 answers.

Specimens from the environmental source samples from every ward in both periods were sampling and cultured for *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae*. Total amount of samples were 823 samples (408 samples in period 1 and 415 samples in period 2).

The environmental sources were

1. Plaster on patient's skin
2. Hand-wash basins (nursing area)
 - 2.1 Sink for hand wash (nursing area)
 - 2.2 Faucet aerators for hand wash (nursing area)
 - 2.3 Bar soap for hand wash or faucet for hand wash solution (nursing area)
3. Equipment wash basin
 - 3.1 Sink for equipment wash
 - 3.2 Faucet aerators for equipment wash
4. Potable water for patients (ward provides)
5. Antiseptic solution

5.1 70% alcohol

5.2 Providine[®] antiseptic solution

5.3 0.9% normal saline solution

6. Patient's mattress

7. Patient's coat (before use)

8. Patient's coat (after use)

9. Air conditioner

10. Flush toilet

Sampling the chemical disinfectants

The specimens from 9 samples (3 diluted chemical disinfectants x 3 samples) of stock diluted chemical disinfectants from wards at Maharat Nakhon Ratchasima Hospital were determined for the efficiency of disinfectants against *Ps. aeruginosa* ATCC 15442.

The efficiency test was repeated again for low efficient disinfectants. The selected disinfectants were freshly prepared by Pharmaceutical Department in Maharat Nakhon Ratchasima Hospital. The efficiency test was performed from the first day until the expired day by 2 days interval (1,3,5,...).

Diluted chemical disinfectants were

Savlon[®] (1.5% chlorhexidine gluconate: 15% cetrimide) was diluted with steriled water in a ratio 1:100.

5 gm. pose-cresol[®] was diluted with 1,000 ml. distilled water.

Sodium hypochlorite was diluted with water from faucet aerator in a ratio 1:20.

3.3 Location of Research

This research was performed at Maharat Nakhon Ratchasima Hospital and Suranaree University of Technology (SUT).

3.4 Instrumentation

Questionnaires

1. The questionnaire for head wards (Questionnaire 1).

1.1 The content validity of the questionnaire 1 had checked by thesis advisors and the experts of Infection Control in the hospital. There were two parts in this questionnaire: general characteristics in the first part and idea about spreading of *Ps. aeruginosa* in ward in the second part.

1.2 The reliability of the questionnaires 1 were tested by head wards at Saint Mary's Hospital.

1.3 The questionnaires 1 were distributed to the head wards of 31 wards at Maharat Nakhon Ratchasima Hospital.

2. The questionnaire for nurses (Questionnaire 2).

2.1 The content validity of the questionnaire 2 had checked by thesis advisors and the experts of Infection Control in the hospital. There were three parts in this questionnaire: general characteristics in the first part and idea about spreading of *Ps. aeruginosa* in wards in the second part and standard precautions of work in the third part.

2.2 The reliability of the questionnaire 2 was tested by 21 nurses in wards at Saint Mary's Hospital.

2.3 The questionnaires 2 were distributed to nurses in 31 wards at Maharat Nakhon Ratchasima Hospital.

3. The specimens from the environment were kept in transport media for transferring to microbiological laboratory to culture for *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* finding.

Specimens were taken in 2 periods (823 samples), which were

3.1 First period

3.1.1 Specimens were taken from the environmental sources in every ward which were 408 samples in total.

3.1.2 The laboratory results about *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae* and the bacterial containing sources found in wards were reported to staffs of each ward.

3.1.3 The knowledges about control and prevention of *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* spreading was given to the nurses in wards.

3.2 There was two months interval for nurses in wards to control and prevent spreading of *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae*.

3.3 Second period

3.3.1 Samples from the same sources as in first period were taken again from every ward. Four hundreds fifteen specimens from these sources were

cultured for *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae*.

3.3.2 The laboratory results about *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae* and the containing sources found in wards were reported to staffs of each ward and informed the head wards and head of Infection Control for control management.

Culture preparation

Specimens were kept in Stuart transport media and transferred to Blood agar plate and Mac Conkey agar plate.

All plates were incubated at 35-37°C for 18-24 hours.

Suspected colonies were grown in trypticase soy broth (TSB) at 35-37°C for 1 hour and then identified by biochemical tests (Donna and Grace, 1975).

Biochemical Tests

Carbohydrate utilization

Bacteria produce acidic products when they ferment certain carbohydrates. The carbohydrate utilization test is designed to detect the change in pH which will occur if fermentation of the given carbohydrate occurs. Acids will lower the pH of the medium causing the pH indicator (phenol red) to turn yellow. If the bacteria do not ferment the carbohydrate then the media remains red. If gas is produced as a by product of fermentation, then the Durham tube will have a bubble in it. The carbohydrate utilization test are the lactose test and the maltose test.

If the media has turned into yellow color this means positive but if it has turned into red the result would be negative and once the bacteria have produced the gas, as by product of fermentation then bubbles inside the tube will be seen (Donna and

Grace, 1975).

Citrate utilization

Citrate utilization test is the test for the ability of bacteria to convert citrate (an intermediate of the Krebs's cycle) to be oxaloacetate (another intermediate of the Krebs's cycle). Citrate is the only carbon source available for the bacteria. If bacteria cannot use citrate then it will not grow. If it can use citrate, then the bacteria grows and the media turns to bright blue as a result of an increase in the pH of the media (Donna and Grace, 1975).

Gelatin utilization

This technique is used to test if bacteria can digest the protein gelatin. To digest gelatin, the bacteria produces an enzyme called gelatinase. To inoculate this media, a transferred needle is used for stabbing the gelatin and transferring the tube into a refrigerator. The tube should be completely chilled prior to observe. If the media is solid after refrigeration then the test is negative (the bacteria does not digest gelatin). If the media is liquefied even after refrigeration, then the test result is positive (Donna and Grace, 1975).

Indole production

Bacteria can break down the amino acid, tryptophan, into indole. TSB is inoculated by a transfer needle. After incubating the bacteria for at least 48 hours, Kovac's reagent is added into the media to detect whether indole has been made by the bacteria. The development of a red/ pink layer on top of the media is a positive result (the bacteria can breakdown tryptophan to form indole). Failure to see a red layer is a negative result (indole is not formed from tryptophan) (Donna and Grace, 1975).

VP (Vogues Proskauer) test

One V-P tablet and 1 ml. of distilled water are placed into a test tube and then inoculate heavily with either 2-3 drops of a heavy suspension of the organism to be tested or with a heavy loopful of organism directly from the plate and incubate at 37°C for 6-8 hours, but not more than 8 hours then refrigerate overnight for incubation. After incubation, add 3 drops of 40% KOH and mix by gently shaking. Next add 2 drops of alpha-naphthol reagent. The reagent will form a thin layer on the surface of the liquid. Allow the tube to stand up to 30 minutes, observe periodically for the appearance of a cherry red color.

In a positive V-P test, the acetoin will react with alpha-naphthol in the alkaline environment provided by the KOH to produce a cherry red color. The color develops first at the surface and then spreads gradually into the lower part of the tube. In a strong reaction the color appears almost immediately but weaker ones may take 20-30 minutes. No color change or the appearance of a copper color is a negative result (Donna and Grace, 1975).

Triple sugar Iron (TSI) test

The purpose of this test is to determine whether organisms can ferment glucose, sucrose and/ or lactose, with or without production of gas. The ability of the organism to produce hydrogen sulphide from thiosulphate in an acid environment is also tested. Fermentation of glucose alone will show yellow colour in the butt of the medium, fermentation of sucrose and/ or lactose will cause both butt and slant to be yellow. Production of hydrogen sulphide leads to blackening. Results are given as slant/ butt/ gas production/ hydrogen sulphide production.

The positive result is A/AG (ferment glucose, lactose and/ or sucrose/ with product of gas result, but negative result is K/K (Non ferment) (Donna and Grace, 1975).

Urea test

This test is used to detect the enzyme urease which break down urea into ammonia. Ammonia is a base and thus will raise the pH of the media if it is present. The pH change will be indicated by a pH indicator called phenol red which is present in the media. A color change from yellow to bright pinkish-red is positive; lack of color change is a negative result (Donna and Grace, 1975).

Catalase test

This test is used to detect the enzyme catalase. This enzyme is responsible for protecting bacteria from hydrogen peroxide (H_2O_2) accumulation, which can occur during aerobic metabolism. If hydrogen peroxide accumulates, it becomes toxic to the organism. Catalase breaks H_2O_2 down into water and O_2 . To perform the catalase test simply smear a small amount of the test organism onto the lid of a Petri plate/culture dish, then add a drop of hydrogen peroxide to the smear. If bubbles become visible (these would be the O_2 bubbling up) then the test is positive and conclude that the organism makes catalase. A lack of bubbles indicates the absence of catalase (Donna and Grace, 1975).

Oxidase test

This tests is used to test for the presence of electron transport system by soaking filter paper disk with oxidase reagent (N,N,N',N'-p-phenylenediamine) and allow to dry. A loop is used to aseptically transfer a large mass of pure bacteria to the disk. The disk is observed for up to 3 minutes. If the area of inoculation turns dark blue

to maroon to almost black, then the result is positive. If a color change does not occur within 3 minutes, the result is negative.

A positive test will result in a color change to pink, through maroon and into black, within 10 to 30 seconds. A negative test will result in a light pink colorization or absence of colorization (Donna and Grace, 1975).

Motility test

The motility test is not a biochemical test. This test is used to check for the ability of bacteria to migrate away from a line of inoculation. To perform this test, the bacterial sample is inoculated into motility media using a needle, then simply stab in the media as a straight line as possible and withdraw the needle very carefully to avoid destroying the straight line. After incubating the sample for 24-48 hours, observation can be made by checking whether the bacteria have migrated away from the original line of inoculation. If migration away from the line of inoculation is shown, it can be concluded that the test organism is motile (positive test). Lack of migration away from the line of inoculation indicates a lack of motility (negative test result) (Donna and Grace, 1975).

Table 1 The results from biochemical tests in this research.

Microorganisms	Carbohydrate utilization	Citrate utilization	Gelatin utilization	Iodole production	VP test	TSI test	Urea test	Catalase test	Oxidase test	Motility test
<i>Ps. aeruginosa</i>	+(g)	nd	+	-	nd	nd	+	nd	+	+
<i>Ps. cepacia</i>	+(-)	nd	+	-	nd	nd	+	nd	+	+
<i>Ps. mallei</i>	+(g)	nd	nd	-	nd	nd	+	nd	+	+
<i>Ps. pseudomallei</i>	+(g)	nd	+	-	nd	nd	+	nd	+	+
<i>Ps. maltophilia</i>	+(-)	nd	+	-	nd	nd	0	nd	+	+
<i>Acinetobacter</i>										
<i>baumannii</i>	+(g)	+	-	-	nd	nd	nd	nd	-	-
<i>Klebsiella</i>										
<i>pneumoniae</i>	nd	+	+	-	+	A/AG	+	nd	nd	-
<i>Escherichia coli</i>						K/A,				
	nd	-	nd	+	-	K /AG	-	nd	-	+,-
<i>Enterobacter</i>										
<i>cloacae</i>	nd	nd	nd	-	+	A/AG	+	+	+	+

Notes

g = gas

0 = no response

nd = not done

4. The efficiency of diluted chemical disinfectants was tested by the AOAC Use-dilution test (Kenneth and Association of Official Analytical Chemists, 1990).

Materials

1, 5 and 10 ml. volumetric pipets

Mohr pipets graduated to 0.1, 1, 5 and 10 ml.

100 ml. measuring cylinder graduated in 1 ml division

20 x 150 mm. bacteriological culture tubes (test culture and subculture tubes)

Water bath was set to $20 \pm 0.2^\circ\text{C}$ for medication tubes

Racks for test tubes

Incubator was set to $37 \pm 0.2^\circ\text{C}$

4 mm. transfer loop (loop was bent at 30 angle with stem) or 10 μl .

Eppendorf (tip ejection) pipet (or equivalent)

100 μl . Eppendorf (tip ejection) pipet

100 ml., 250 ml. beakers

15 x 110 mm. petri dishes: filter paper (Whatman No.2) was placed into the petri dishes and sterilized by autoclave at 121°C for 15 min.

25 x 150 mm. pyrex test tubes for medication tubes

Carriers: polished stainless steel cylinders (Penicillin cups) size 8 mm., 6 mm., length of cup 10 mm.

All glasswares were sterilized for 2 hours at 180°C

Sterilized distilled water

1N sodium hydroxide solution (use for carriers cleaning)

0.1% asparagin solution (in distilled water)

Forcep and transfer loops

Organisms preparation

The bacteria that used for testing was *Ps. aeruginosa* ATCC 15442. They were cultured for 48-54 hours at 37°C in 10 ml. liquid media. Bacteria were then collected and put into a beaker size 100 ml. Amount for testing was about 20 ml.

Carriers preparing

Cleaned carriers (10 carriers per one bacteria) were put into 1N sodium hydroxide overnight before washing them by distilled water for many times. Let the carriers dried and then put them into 0.1% asparagin solution and sterilized at 121°C for 15 min.

Use-Dilution method

1. All 10 carriers were transferred into the beaker containing bacteria. After 10 to 15 minutes, the forceps were then used to remove each carrier, put it on the filter paper that was on the bottom of the sterilized petri dish in vertical position with the same distance between each of them. They were then incubated at 37°C for 20 to 60 minutes.

2. Each sample of diluted chemical disinfectants was prepared and used for testing. Ten tubes were used per 1 sample and each tube contained 5 ml. of sample.

3. The prepared carriers were put into tube dilution (1 carrier per 1 tube). The timer was started when the first carrier was put into the first tube, the next carrier was put into the following tube by 1 minute apart until finished. After 10 minutes, each carrier, which was in diluted disinfectant tube, was transferred into subculture media by using holdfast hooked the carrier. The first carrier was transferred first, followed by the rest by 1 minute apart until finished.

4. The carriers were cultured in subculture tubes for 48 hours at 37°C.

5. Turbidity in the subculture tubes was observed and reported as the positive results.

Evaluation

1. Bacterial growth must not be shown in any of the ten subculture tubes.
2. If the growth of bacteria was found in even one tube, indicating that dilution in the group of samples did not safe enough to kill the bacteria.

3.5 Data Collections

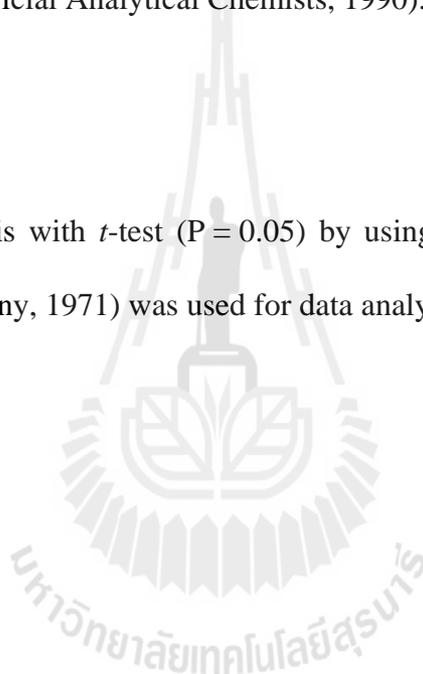
1. The thesis proposal was sent to the board of directors of Maharat Nakhon Ratchasima Hospital for permission.
2. The thesis proposal was sent to the head of Infection Control and the head wards at Maharat Nakhon Ratchasima Hospital to inform about the research.
3. Questionnaires were distributed and collected from head wards and nurses in wards.
4. Specimens were collected in transport media for transferring to microbiological laboratory for bacterial identification.
5. The laboratory results about *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae* and the bacterial containing sources found in wards were reported to nurses in wards.
6. The knowledges about standard precautions of work, control and prevention of *Pseudomonas* spp. spreading in the ward were given to nurses in wards.
7. After 2 months, specimens from the same sources as the first period were collected in transport media for transferring to microbiological laboratory for bacterial identification.

8. The laboratory results about *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae* and the bacterial containing sources found in wards were reported to the experts of Infection Control, head wards and nurses in wards for control and prevention management.

9. The efficiency of diluted chemical disinfectants using in wards at Maharat Nakhon Ratchasima Hospital was tested by the AOAC Use-dilution test (Kenneth and Association of Official Analytical Chemists, 1990).

3.6 Data Analysis

Statistical analysis with *t*-test ($P = 0.05$) by using the SPSS for Window V.15 software program (Finny, 1971) was used for data analysis.



CHAPTER IV

RESULTS AND DISCUSSION

4.1 Results

Pseudomonas spp. in wards at Maharat Nakhon Ratchasima Hospital were studied to compare number of species of microorganisms cultured from the environmental sources before and after giving knowledges to the nurses. Species of *Pseudomonas* that associated with human diseases from the environments, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* in the environmental sources and bactericidal efficiency of diluted chemical disinfectants used in wards were studied. The results were presented into 3 parts as follows:

1. The information taken from questionnaires.

- 1.1 General characteristics of head wards and nurses in wards at Maharat Nakhon Ratchasima Hospital.

- 1.2 Knowledges about spreading of *Ps. aeruginosa* in wards at Maharat Nakhon Ratchasima Hospital.

- 1.3 Standard precaution procedures for nurses in wards at Maharat Nakhon Ratchasima Hospital.

2. Microorganisms from the environmental sources in wards at Maharat Nakhon Ratchasima Hospital.

3. The bactericidal efficiency of diluted chemical disinfectants which were used in wards at Maharat Nakhon Ratchasima Hospital.

1. The information taken from questionnaires.

1.1 General characteristics of head wards and nurses in wards at Maharat Nakhon Ratchasima Hospital.

Table 2 Socio-demographic characteristics of head wards and nurses in wards at Maharat Nakhon Ratchasima Hospital.

Characteristics	Head wards (n = 31)		Nurses in wards (n = 208)	
	Number	Percentage	Number	Percentage
Age group (years)				
21-30	0	0	70	33.65
31-40	2	6.45	74	35.58
41-50	18	58.07	43	20.67
51-60	11	35.48	21	10.10
mean ± S.D.	47.71 ± 4.28		35.87 ± 9.06	
min - max	38 - 56		20 - 59	
Classified				
Registered nurse	31	100	189	90.87
Technical nurse	0	0	19	9.13
Gender				
Male	0	0	5	2.40
Female	31	100	203	97.60

The studied subjects included 31 head wards and 208 nurses in wards at Maharat Nakhon Ratchasima Hospital. The distribution of studied subjects by age, classified and gender were shown in Table 2.

Most of head wards were 41 to 50 years (the mean age was 47.71 years). All 31 head wards were registered nurses and female. Most of nurses in wards were 31 to 40 years (the mean age was 35.87 years). Among 208 nurses in wards, 90.87% were registered nurses and 9.31% were technical nurses. 97.60% of nurses in wards were female and 2.40% were male.

1.2 Knowledges about spreading of *Ps. aeruginosa* in wards at Maharat Nakhon Ratchasima Hospital.

Table 3 Knowledges about spreading of *Ps. aeruginosa* in wards at Maharat Nakhon Ratchasima Hospital. (Head wards, n = 31) (Nurses in wards, n = 208)

Knowledges about spreading	Head wards (n = 31)				Nurses in wards (n = 208)			
	Yes	%	No	%	Yes	%	No	%
1. Do you know what <i>Pseudomonas</i> spp. is?	27	88.10	4	12.90	185	88.94	23	11.06
2. Did <i>Ps. aeruginosa</i> cause antibiotic resistant at Maharat Nakhon Ratchasima Hospital?	25	80.65	6	19.35	174	83.65	34	16.35
3. Do <i>Pseudomonas</i> spp. grow in water?	27	87.10	4	12.90	175	84.13	33	15.87

Table 3 (Continued)

Knowledges about spreading	Head wards (n = 31)				Nurses in wards (n = 208)			
	Yes	%	No	%	Yes	%	No	%
4. Did mowing and cutting trees effect on spreading of <i>Pseudomonas</i> spp. in the hospital?	12	38.71	19	61.29	105	50.48	103	49.52
5. Did the sink cause spreading of antibiotic resistant gram negative bacilli bacteria?	27	87.10	8	25.81	180	86.54	28	13.46
6. Did you think staff's sinks are clean enough?	20	64.52	11	35.48	159	76.44	49	23.56
7. Have your staffs ever cleaned the medical equipments in the hand wash sinks?	11	35.48	20	64.52	105	50.48	103	49.52
8. Have your staffs ever washed their hands before nursing care for each patient at the medical equipment sinks or cooking sinks?	9	29.03	22	70.97	62	29.81	146	70.19
10. When the patients are discharged from the hospital, do your staffs change the bed sheets immediately?	28	90.32	3	9.68	172	82.69	36	17.31

Table 3 (Continued)

Knowledges about spreading	Head wards (n = 31)				Nurses in wards (n = 208)			
	Yes	%	No	%	Yes	%	No	%
11. Did your staffs change the bed sheets immediately when it was found secretions or blood?	29	93.55	2	6.45	169	81.25	39	18.75
12. When the nurse found dirt or dust contaminations, did your staffs change the bed sheets immediately?	23	74.20	8	25.80	142	68.27	66	31.73
13. Before changing the bed sheet, did your staffs clean the mattress everytime?	12	38.71	19	61.29	110	52.88	98	47.12

Details of knowledges about spreading of *Ps. aeruginosa* were shown in Table 3. It was found that most of head wards and nurses in wards had high percentage of knowledges about spreading of *Ps. aeruginosa*, but had low percentage in head wards that staffs of wards always clean the mattress before changing the bed sheet (38.71%).

1.3 Standard precaution procedures for working nurses at Maharat Nakhon Ratchasima Hospital.

Table 4 Standard precautions of working nurses at Maharat Nakhon Ratchasima Hospital. (Questionnaire 2, n = 208)

Standard precautions for working nurses	Everytime		Sometimes		Never	
	Number	%	Number	%	Number	%
1. You often wash your hands before nursing care for each patient.	146	70.19	62	29.81	0	0
2. You have ever wash your hands after nursing care for each patient.	177	85.10	31	14.90	0	0
3. You often wash your hands with antiseptic.	167	80.29	41	19.71	0	0
4. If your gloves tear, you will change them.	199	95.67	9	4.33	0	0
5. You always wear gloves while you are taking care of patients.	72	34.62	136	65.38	0	0
6. You always wear gloves when you have to touch patient's blood or secretion.	164	78.85	44	21.15	0	0
7. You wash your hands after taking off your gloves.	19	9.13	19	9.13	170	81.73

Table 4 (Continued)

Standard precautions for working nurses	Everytime		Sometimes		Never	
	Number	%	Number	%	Number	%
8. You change your gloves when you make a new activity.	176	84.62	23	11.06	9	4.33
9. You wear a needle sheath by one hand.	132	63.46	76	36.54	0	0
10. You cut drug ampule by using cover gauze.	68	32.69	137	65.87	3	1.44
11. You always wear mask before exposing patients.	63	30.29	132	63.46	13	6.25
12. You always take a mask at neck when you finish your activity.	120	57.69	76	36.54	12	5.80
13. You will change mask when it wet.	187	89.90	17	8.17	4	1.92
14. You always wear the accessories (gloves, mask, apron, eye-glass, etc.) for protecting body before exposing patients.	63	30.29	125	60.10	20	9.62
15. You always suggest to patients or their family members to take part in prevention themselves about infection in ward.	36	17.31	160	76.92	12	5.77

Details of standard precaution practices were shown in Table 4. It was found that most of the working nurses had high percentage of nursing care on standard precautions, but had low percentage in washing their hands after taking off their gloves (9.13 %) and always suggest patients or their family members to take part in preventing themselves from infection in wards (17.31 %).

Table 5 Standard precaution practice levels of working nurses at Maharat Nakhon Ratchasima Hospital (n = 208)

Standard precaution levels	Number	%
High level (score 24-30)	114	54.81
Medium level (score 18-23)	88	42.31
Low level (score <18)	6	2.88
mean \pm S.D. = 23.6 \pm 2.42		min-max = 17-29

Scores of standard precaution for each question were

Everytime = 2 score

Sometimes = 1 score

Never = 0 score

The standard precaution procedures for working nurses at Maharat Nakhon Ratchasima Hospital were at (54.81%) high level, and only 2.88% of working nurses had low level which was shown in table 5.

2. Microorganisms from the environmental sources in wards at Maharat Nakhon Ratchasima Hospital.

Table 6 The present of *Pseudomonas* spp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* in specimens sampled from several environmental sources before and after giving the knowledges in wards at Maharat Nakhon Ratchasima Hospital.

The enviromental sources	<i>Ps.aeruginosa</i>	<i>Ps.cepacia</i>	<i>Ps.mallei</i>	<i>Ps.pseudomallei</i>	<i>Ps.maltophilia</i>	<i>Acinetobacter baumannii</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Enterobacter cloacae</i>	Total
1. Plaster on Patient's skin.										
Before	0	0	0	0	0	1	0	0	0	1
After	0	0	0	0	0	0	0	0	0	0
2. Sink for hand wash (nursing area).										
Before	8	0	0	0	7	8	7	1	1	32
After	9	0	0	0	3	8	2	2	2	26

Notes

408 samples in period 1, 415 samples in period 2.

Table 6 (Continued)

The enviromental sources	<i>Ps.aeruginosa</i>	<i>Ps.cepacia</i>	<i>Ps.mallei</i>	<i>Ps.pseudomallei</i>	<i>Ps.maltophilia</i>	<i>Acinetobacter baumannii</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Enterobacter cloacae</i>	Total
3. Faucet aerators for hand wash (nursing area).										
Before	2	0	0	0	6	10	4	1	3	26
After	3	0	0	0	3	10	1	1	0	18
4. Bar soap for hand wash or faucet for hand wash solution (nursing area).										
Before	2	0	0	0	1	1	0	0	0	4
After	3	0	0	0	0	1	2	0	3	9
5. Sink for equipment wash.										
Before	5	0	0	0	3	7	3	5	1	24
After	4	0	0	0	1	12	8	2	1	28

Notes

408 samples in period 1, 415 samples in period 2.

Table 6 (Continued)

The enviromental sources	<i>Ps.aeruginosa</i>	<i>Ps.cepacia</i>	<i>Ps.mallei</i>	<i>Ps.pseudomallei</i>	<i>Ps.maltophilia</i>	<i>Acinetobacter baumannii</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Enterobacter cloacae</i>	Total
6. Faucet aerators for equipment wash.										
Before	2	0	0	0	5	9	1	2	3	22
After	4	0	0	0	1	9	2	0	0	16
7. Potable water for patients (ward provides).										
Before	1	0	0	0	0	0	0	0	0	1
After	0	0	0	0	0	2	0	0	0	2
8. 70% alcohol (treatment unit).										
Before	0	0	0	0	0	0	0	0	0	0
After	0	0	0	0	0	0	0	0	0	0

Notes

408 samples in period 1, 415 samples in period 2.

Table 6 (Continued)

The enviromental sources	<i>Ps.aeruginosa</i>	<i>Ps.cepacia</i>	<i>Ps.mallei</i>	<i>Ps.pseudomallei</i>	<i>Ps.maltophilia</i>	<i>Acinetobacter baumannii</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Enterobacter cloacae</i>	Total
9. Providine® antiseptic solution (treatment unit).										
Before	0	0	0	0	0	0	0	0	0	0
After	0	0	0	0	0	0	0	0	0	0
10. 0.9% normal saline solution (treatment unit).										
Before	0	0	0	0	0	0	0	0	0	0
After	1	0	0	0	0	1	0	0	0	2
11. Patient's mattress.										
Before	0	0	0	0	0	1	0	0	0	1
After	0	0	0	0	0	3	1	0	0	4
12. Patient's coat (before use).										
Before	0	0	0	0	0	0	0	0	0	0
After	0	0	0	0	0	1	0	0	0	1

Notes

408 samples in period 1, 415 samples in period 2.

Table 6 (Continued)

The enviromental sources	<i>Ps.aeruginosa</i>	<i>Ps.cepacia</i>	<i>Ps.mallei</i>	<i>Ps.pseudomallei</i>	<i>Ps.maltophilia</i>	<i>Acinetobacter baumannii</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Enterobacter cloacae</i>	Total
13. Patient's coat (after use).										
Before	1	0	0	0	0	1	0	1	0	3
After	1	0	0	0	0	0	0	2	0	3
14. Air conditioner.										
Before	1	0	0	0	0	1	0	0	0	2
After	1	0	0	0	0	0	0	0	0	1
15. Flush toilet.										
Before	3	0	0	0	2	9	2	3	1	20
After	4	0	0	0	0	7	3	5	1	20
Total										
Before	25	0	0	0	24	48	17	13	9	136
After	30	0	0	0	8	54	19	12	7	130

Notes

408 samples in period 1, 415 samples in period 2.

Microorganisms which were cultured from the environmental sources before (period 1) and after (period 2) giving the knowledges in wards at Maharat Nakhon Ratchasima Hospital were shown in Table 6. The highest number of species of microorganisms was *Acinetobacter baumannii* which was found in 48 specimens in period 1, 54 specimens in period 2, *Ps. aeruginosa* found in 25 specimens in period 1, 30 specimens in period 2 and *Klebsiella pneumoniae* found in 17 specimens in period 1, 19 specimens in period 2. The environmental source which was found the most of microorganisms was the sink for hand wash (nursing area), 32 specimens in period 1 and 26 specimens in period 2. The second was sink for equipment wash, 24 specimens in period 1, 28 specimens in period 2 and the faucet aerators for hand wash, 26 specimens in period 1 and 18 specimens in period 2.

The number of species of *Ps. aeruginosa*, *Ps. maltophilia*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* that were cultured from the environmental sources before and after educating about the control and prevention of nosocomial infection ($p = 0.42, 0.44, 0.45, 0.37, 0.26$ and 0.39 respectively) found that **no** significantly differences in these two groups ($P < 0.05$). In addition *Ps. cepacia*, *Ps. mallei* and *Ps. pseudomallei* were not found in both before and after education.

3. The bactericidal efficiency of diluted chemical disinfectants used in wards at Maharat Nakhon Ratchasima Hospital.

Table 7 The bactericidal efficiency of diluted chemical disinfectants used in wards at Maharat Nakhon Ratchasima Hospital.

Diluted chemical disinfectants	Lab Results			
	positive samples / total samples		Date	
	24 hours	48 hours	Made	Expired
Savlon® 1:100				
Sample 1	3/10	4/10	25/9/2551	2/10/2551
Sample 2	6/10	6/10	28/9/2551	5/10/2551
Sample 3	8/10	8/10	25/9/2551	2/10/2551
Pose-cresol®				
Sample 1	8/10	8/10	18/9/2551	18/10/2551
Sample 2	0/10	6/10	22/9/2551	22/10/2551
Sample 3	0/10	6/10	22/9/2551	22/10/2551
Sodium hypochlorite 1:20				
Sample 1	0/10	0/10	1/10/2551	29/10/2551
Sample 2	0/10	0/10	1/10/2551	29/10/2551
Sample 3	0/10	0/10	1/10/2551	29/10/2551

Table 7 showed that sodium hypochlorite (1:20) was the most effective disinfectant against *Ps. aeruginosa* ATCC 15442. While savlon® (1:100) and pose-cresol® did not pass the criteria because *Ps. aeruginosa* ATCC 15442 was found in some tubes of ten tubes within 48 hours, indicating that both diluted chemical

disinfectants could not kill the *Ps. aeruginosa* ATCC 15442 even before the expiration date.

Table 8 The bactericidal efficiency of diluted chemical disinfectants with low efficiency from Pharmaceutical Department of Maharat Nakhon Ratchasima Hospital.

Diluted chemical disinfectants	Lab Results	
	positive samples / total samples	
	24 hours	48 hours
Savlon [®] 1:100		
day 0	0/10	0/10
day 3	7/10	8/10
pose-cresol [®]		
day 0	10/10	10/10

The efficiency tests were shown that savlon[®] (1:100) could kill *Ps. aeruginosa* ATCC 15442 within 48 hours only but pose-cresol[®] could not possibly kill *Ps. aeruginosa* ATCC 15442 even within 24 hours, suggesting that both savlon[®] (1:100) and pose-cresol[®] had very poor efficiency, they did not work efficiently as it stated in the manufactory details. The efficiency of diluted chemical disinfectants were shown in Table 8.

4.2 Discussion

From this research, the results were found very positive and interesting indeed. Results of the findings were as following:

In questionnaire part, the informations from the group of head wards, almost all of them had an average age between 41-50 years old because the higher position was selected by the working experience. Meanwhile, nurses in wards were around 21-40 years old. The Bachelor degree in Nursing Science was required for the head ward position, while nurses in wards were registered nurses (90.87%) and technical nurses (9.13%). Most of nurses in both groups were female, 97% for nurses in wards and 100% for head wards. The research was conducted by the idea about the epidemic of *Ps. aeruginosa* in wards at Maharat Nakhon Ratchasima Hospital. Results of this study suggested that attitudes of both head wards and nurses in wards were correlated with the epidemic of *Ps. aeruginosa* in nearly the same high level. On the other hand, the idea about the spreading of *Ps. aeruginosa* by lawn mower with this point has shown that unknowing of the epidemic by head wards were 61.29%, which were higher than nurses in wards which the unknowing was only 49.52%. When the knowing of spreading out by water was better, head wards had 87.10% and nurses in wards had 84.13%. Moreover, most of nurses (both head wards and nurses in wards) had known very well about the epidemic of *Ps. aeruginosa*, which was mediated through the water but did not realize that it could also be mediated by soil and environmental. Informations from the questionnaires about spreading of microorganisms from head wards were different from nurses in wards as below:

1. Nurses in wards thought that ward staffs had ever cleaned the medical equipments in hand wash sinks 50.48% while head wards thought only 35.48%.

2. The idea that ward staffs had ever washed their hands at the medical equipment sinks or cooking sinks before nursing care for each patient was not quite different, 29.03% in head wards and 29.81% in nurses in wards.

3. The idea that ward staffs did not change the bed sheets when they had gotten dirty by dust and dirt. There were 25.8% in head wards and 31.73% in nurses in wards.

4. The idea that ward staffs did not regularly sanitize the beds before changing the sheet were 61.29% in head wards and 47.12% in nurses in wards.

All practices above had affected spreading out of *Ps. aeruginosa* such as microorganisms from medical appliances could contaminate in the staff's sinks. Without washing their hands before and after the nursing care could cause getting diseases from whom that used the sink and was able to pass microorganisms to patients. Moreover, 29.03% of head wards and 29.81% of nurses in wards thought that their staffs were still used medical equipment sinks or cooking sinks to wash their hands before nursing care which was a condition for microorganisms spreading as well. The amount of bacterial species causing diseases was found around staff's sinks which were 32 spp. in period 1 and 26 spp. in period 2, their taps were 26 spp. in period 1 and 18 spp. in period 2 as well as around equipment sinks were 24 spp. in period 1 and 28 spp. in period 2, their taps were 22 spp. in period 1 and 16 spp. in period 2. Every kind of screened bacteria except *Ps. cepacia*, *Ps. mallei* and *Ps. pseudomallei* was found. *Ps. cepacia* and *Ps. mallei* are not found in Thailand (Selina, Online, 2007). *Ps. cepacia* seldom infects humans. *Ps. mallei* causes glanders, a serious infectious disease of animals (primarily horses, although it also has been isolated in donkeys, mules, goats, dogs, and cats). Transmission is believed to occur

through direct contact. Glanders transmission to humans is rare and presumably occurs through inoculation of broken skin or the nasal mucosa with contaminated discharges. *Ps. pseudomallei* is endemic in Southeast Asia, but infections outside this area are uncommon (Selina, Online, 2007). *Ps. aeruginosa* and *Ps. maltophilia* that associated with human diseases were isolated from the environment in wards at Maharat Nakhon Ratchasima Hospital.

Acinetobacter baumannii and *Klebsiella pneumoniae* were found in mattress due to humidity and dust from environment in hospital (Pattarachai, 2006). The mattresses are significant bacterial accumulated sources that contact directly to the patients and bacteria can easily spread to patients. Another important factor was the patient's clothes that could be the sources of bacteria. Thus, cleaning the mattresses, sheets and clothes are necessary.

From the specimens of environmental sources, *Acinetobacter baumannii* was found just once. Besides, *Ps. aeruginosa*, *Acinetobacter baumannii* and *Escherichia coli* were found in the patient's clothes after wearing. If the lying was not good enough and had overflow from the bucket or unwearing the gloves to collect the patient's clothes, that would be the cause that made these bacteria to spread out. The other cause that should not be ignored was plasters that stucked with the wound patients. Humidity also be the cause that made *Acinetobacter baumannii* could be found.

Ps. aeruginosa and *Acinetobacter baumannii* in the water, which was prepared for patients and relatives and also the staffs in some wards, were also observed. From observation and questioning the nurses in wards, it was found that they used the glasses in the wards that have been prepared for relatives of patients to

fetch from the tank of the filtered drinking water and did not turn on the hydrant from that tank that had been prepared. This was a cause of the contamination of water because of dirty hands and dirty glasses. If these bacteria entered the gastrointestinal tract, it would cause the tract infection (Sopon, 1981).

“Sterilizing solution”, such as 70% alcohol, providine[®] antiseptic solution and 0.9% normal saline solution, is the solution that must not be possibly found the bacteria. Surprisingly, in this study, bacteria (*Ps. aeruginosa* and *Acinetobacter baumannii*) was found in 0.9% normal saline solution. This solution are usually used for wound cleaning, locking the blood vessel to give the medicine through the vessel. By observing and interviewing the nurses, it was found that they have used 0.9% normal saline solution for over than 24 hours after opening the bottles due to the night shift nurses who had prepared the treatment cart and used it again from the past schedule without specify the opened time and the expired time. Moreover, the researcher discovered that the 0.9% normal saline solution bottle had been punctured by the syringe and it was still remaining on the rubber cork. Which would let the bacteria could pass through the bottle if it was contaminated. When the contaminated one was used with the patients, it would cause the wound and the blood vessels to be infected in the other way (Pattarachai, 2006).

After comparing the amount of the bacteria which were in the environmental sources before and after giving knowledges to nurses about control and prevention of microorganisms spreading, there was no significant difference between these sources groups ($P < 0.05$).

In studied case about standard precautions of nurses in wards were found 54.81% at high rate and 42.31% at medium rate which was nearly the same and 2.88%

at low level. These findings indicated that standard precautions of nurses in wards were not well comprehensive, that would be the important part to raise the amount of bacteria in the environment. Before and after giving knowledges to the nurses about “standard precautions, control and prevention”, the bacteria in the environmental sources were not different. The reasons that nurses did not aware towards standard precautions may be due to over workload, over confidence and ignorance that were reported by several studies (Conly, Hill, Ross, Lertzman, and Louie, 1978).

The further information taken from questionnaires from nurses in wards were 1) wearing a mask before exposing patients everytime (30.29%), sometimes (63.46%) and did not equip (6.25%), and 2) always wearing the accessories (gloves, mask, apron, eye-glass, etc.) before give nursing care for each patient (30.29%), sometimes (60.1%) and did not equip (9.62%). Moreover, the researcher had found that how to convince nurses to strict to the standard precautions was very important. Nurses in wards did not regularly practice. Although, the record was not shown that they ignored to practice but they occasionally practice, this could also be the factor that made the bacteria easily spread by the nurses in wards to other patients.

Within the wards that had different diseases and symptoms, it could make the bacteria spread through other patients. It was found that the nurses washed their hands before nursing care for each patient for sometimes (29.81%), washed their hands after nursing care for each patient for sometimes (14.90%) and used the gloves when they had to touch patient’s blood or secretion for sometimes (21.15%). Besides, there was a very important information that the percentages of sometimes practice was higher than always practice. The using of gloves when nursing care to patients was found that the sometimes practice was at high rate as 65.38% while always practice was

only 34.62%, As well as cutting drug ampule by not using cover gauze was 1.44% and 65.87% for sometimes, so the bacteria could contaminate the syringe.

For the bactericidal efficiency test for 3 types of the disinfectant solutions using within the wards, each type had 3 samples, it was found that the only solution that passed the criterion that could sterilize the *Ps. aeruginosa* ATCC 15442 was sodium hypochlorite (1:20). Other solutions, savlon[®] (1:100) and pose-cresol[®] did not pass the criteria because the infection of *Ps. aeruginosa* ATCC 15442 was found in some tubes of ten tubes within 48 hours, indicating that both diluted chemical disinfectants could not kill the *Ps. aeruginosa* ATCC 15442 even before expired date period. Therefore, the test was repeated again for these poor efficient solutions. It was found that savlon[®] (1:100) could kill the *Ps. aeruginosa* ATCC 15442 but it was not exceed 48 hours after application. It passed the criteria within 48 hours but it did not pass the criteria after 48 hours while it had stated to expire within 7 days. For the pose-cresol[®], it produced a very poor result indeed. It was unable to kill *Ps. aeruginosa* ATCC 15442 within 24 hours and the time it had stated to be expire was 30 days.

The results came to the conclusion of these tests that both savlon[®] (1:100) and pose-cresol[®] had very poor efficiency, they did not work efficiently as it stated in the manufactory details. If it was continued to be used at the same concentration in the hospital it could be danger to risk of spreading the bacteria from one patient to another. Our findings matched to the test results from Sappasittiprasong Ubolratchathani Hospital, savlon[®] (1:100) did not pass the criteria and they explained that it might be contaminated during the dilution process of savlon[®] (1:100) in Pharmaceutical Department. Eight lot (57.40%) out of fourteen lots of diluted

chemical disinfectants used in hospital were contaminated (Suppasittipasong Ubonratchanee Hospital, 2536). At Maharat Nakhon Ratchasima Hospital, savlon[®] (1:100) is usually used to flush for cleaning the sexual organ, both before and after putting the urethral catheter and cleaning the sexual organ before pelvic examination and pose-cresol[®] is normally used for soaking clinical thermometer. The causes of errors in such standard dilution of chemical disinfectants to be used in hospital could be possibly due to their low quality, low constant or over dilution for saving the cost. Out of standard disinfectant solution that was produced by the company could also be the important part in the quality of the chemical disinfectants that were diluted and used in hospitals. Other factors, the mixing container and the environment around that area were also important. By using the low quality chemical disinfectants, there was a high risk rate because it was not only harmful to the patients directly but it also caused nosocomial infections and it could be spread outside the hospitals later on. The effectiveness of the chemical disinfectants use in the hospitals has been concerned for many years. One hundreds and two samples of the chemical disinfectants from 32 hospitals throughout the country were tested for their disinfectants qualities. The results showed that 43.1% samples were out of standard. (Department of Medical Sciences Center of Thailand, 2536). Thus, the chemical disinfectants which were out of standard, were one of the harmful reasons for nosocomial infections.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This research used the questionnaires for data collection from 31 head wards and 208 nurses in wards at Maharat Nakhon Ratchasima Hospital. Attitudes of head wards and nurses in wards were correlated with the epidemic of *Ps. aeruginosa* in nearly the same high percentage. The standard precaution practices for working nurses were 54.81% at high level. In addition, collected samples from the environmental sources were analyzed for the number of species of microorganisms found in the wards in 2 periods with 2 months interval which were before and after giving knowledges to nurses about standard precautions, control and prevention of microorganisms spreading. Results of the findings are as follow

1. In the comparison between the amount of the bacteria found in the environmental sources before and after giving the knowledges to nurses about control and prevention of microorganisms spreading, there was no significant differences in these selected sources groups.

2. The species of *Pseudomonas* found in the environmental sources in common wards at Maharat Nakhon Ratchasima Hospital were *Ps. aeruginosa* and *Ps. maltophilia*. These *Pseudomonas* spp. are associated with human diseases.

3. Microorganisms were found in every selected environmental source, except in 70% alcohol and providine[®] antiseptic solution in common wards at Maharat Nakhon Ratchasima Hospital.

From the bactericidal efficiency test of diluted chemical disinfectants used in wards at Maharat Nakhon Ratchasima Hospital, the only chemical disinfectant that passed the criterion which was able to kill the *Ps. aeruginosa* ATCC 15442 was sodium hypochlorite (1:20) while savlon[®] (1:100) and pose-cresol[®] did not pass the criteria. The tests were repeated again for the low efficiency chemical disinfectants, savlon[®] (1:100) and pose-cresol[®]. These two chemical disinfectants were diluted by Pharmaceutical Department at Maharat Nakhon Ratchasima Hospital and tested for bactericidal efficiency from the first day until the expired day by 2 days interval (1,3,5,...). The efficiency test results showed that only savlon[®] (1:100) was able to pass the criteria within 48 hours after application. However, its bactericidal effects on *Ps. aeruginosa* ATCC 15442 did not pass the criteria after 48 hours, indicating that this diluted disinfectant did not work efficiently as it stated in the manufactory efficiency rate of 7 days. Furthermore, the pose-cresol[®] had produced a very poor result indeed. It was not able to kill *Ps. aeruginosa* ATCC 15442 within 24 hours and the time that it had stated to be expired was 30 days.

5.2 Recommendation

1. Recommendation from the findings of this study.

1.1 The information about sources of microorganisms which were yet found in wards will be reported to the head of Infection Control for control and prevention management.

1.2 Improvement the knowledges about standard precautions, control and prevention of microorganisms spreading can be done by having a short course training program for nurses.

1.3 The findings about microorganisms found in environmental sources in wards should make awareness to nurses for strict precautions not to spread pathogens to patients.

1.4 Random sampling the diluted chemical disinfectants used in wards at Maharat Nakhon Ratchasima Hospital should be frequently checked for their bactericidal efficiency properties.

2. Future studies should be aims to

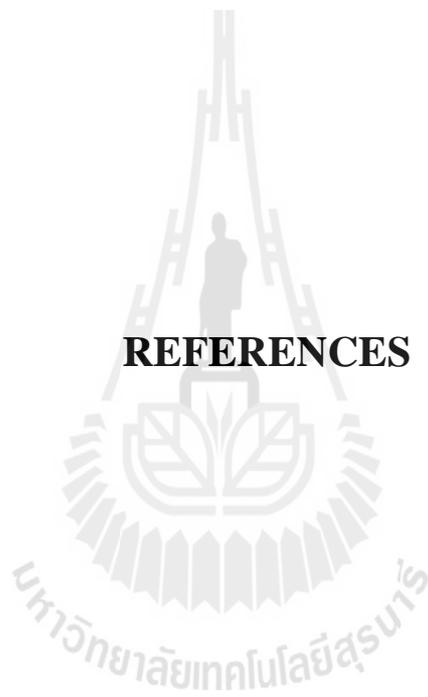
2.1 Study incidence or prevalence of other nosocomial infections and preventable risks.

2.2 Study other microorganisms from other environmental sources in wards at Maharat Nakhon Ratchasima Hospital.

2.3 Test the bactericidal efficiency of other disinfectants which were used in wards at Maharat Nakhon Ratchasima Hospital.

2.4 Study the cause of risk factor for microorganisms spreading in wards at hospital.

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APPENDICES

APPENDIX A
BACTERIAL CULTURE PREPARATION



APPENDIX A

BACTERIAL CULTURE PREPARATION

Media

Blood agar plate

Ingredients (gm/L)

Blood agar base medium	500.00	ml.
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Sterile blood	20.00	ml.
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Carbohydrate utilization

Ingredients (gm/L)

Dipotassium phosphate	0.50	gm.
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Sodium chloride	0.20	gm.
-----------------	------	-----

Magnesium sulphate	0.20	gm.
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Calcium carbonate	1.00	gm.
-------------------	------	-----

Carbohydrate	1.00	gm.
--------------	------	-----

Bromthymol blue (0.5 percent alcoholic)	5.00	gm.
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Yeast extract, Bacto	0.50	gm.
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Potassium nitrate	0.50	gm.
-------------------	------	-----

Agar (bacto)	15.00	gm.
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Distilled water	1,000.00	ml.
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Catalase test

Ingredients (gm/L)

Hydrogen peroxide 30%	100.00	ml.
Acetophenetidine	900.00	ml.

Citrate utilization

Ingredients (gm/L)

Ammonium sulphate	0.50	gm.
Sodium nitrate	0.50	gm.
Magnesium sulphate	0.50	gm.
Dipotassium phosphate	0.50	gm.
Calcium chloride	0.20	gm.
Ferric ammonium citrate	10.00	gm.
Agar	15.00	gm.

Gelatin utilization

Ingredients (gm/L)

Peptic digest of animal tissue	25.00	gm.
Meat extract	7.50	gm.
Sodium chloride	5.00	gm.
Gelatin	120.00	gm.
Ferrous chloride	0.50	gm.
Agar	1.00	gm.

Indole production

Ingredients (gm/L)

<i>p</i> -dimethyl aminobenzaldehyde	10.00	gm.
Amyl alcohol	150.00	ml.
Hydrochloric acid (concentrated)	50.00	ml.

Mac Conkey agar plate

Ingredients (gm/L)

Peptone	17.00	gm.
Proteose peptone	3.00	gm.
Lactose	10.00	gm.
Bile salts	1.50	gm.
Sodium chloride	5.00	gm.
Agar	1.35	gm.
Neutral red	0.03	gm.
Crystal violet	0.01	gm.
Distilled water	1,000.00	ml.

Motility test

Ingredients (gm/L)

Beaf extract	3.00	gm.
Pencreatic digest of casein	10.00	gm.
Agar	4.00	gm.

Oxidase test

Ingredients (gm/100 ml)

Dihydrochloride	0.60	gm.
Stabilizing agent	0.02	gm.
Dimethyl sulfoxide (DMSO)	100.00	ml.

Stuart transport media

Ingredients (gm/L)

Sodium glycerophosphate	10.00	gm.
Sodium thioglycolate	1.00	gm.
Calcium chloride	0.10	gm.
Methylene blue	0.002	gm.
Agar	3.00	gm.
Final pH 7.4 ± 0.2		

Trypticase soy broth

Ingredients (gm/L)

Pancreatic digest of casein	17.00	gm.
Enzymatic soy digest	3.00	gm.
Sodium chloride	5.00	gm.
Dextrose	2.50	gm.
Dipotassium phosphate	2.50	gm.
Final pH 7.3 ± 0.2		

Urea test

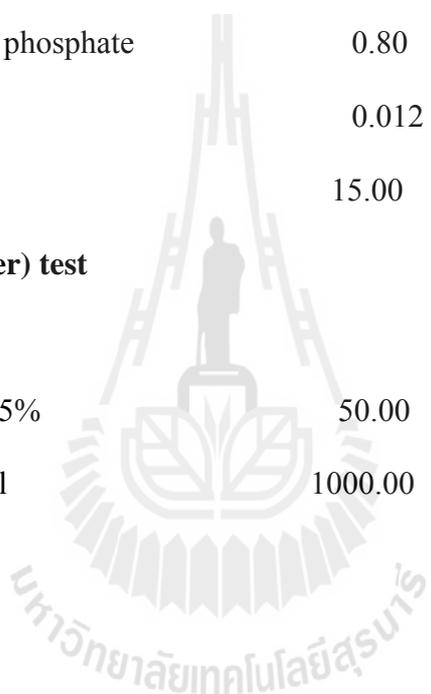
Ingredients (gm/L)

Peptic digest of animal tissue	1.00	gm.
Dextrose	1.00	gm.
Sodium chloride	5.00	gm.
Disodium phosphate	1.20	gm.
Monopotassium phosphate	0.80	gm.
Phenol red	0.012	gm.
Agar	15.00	gm.

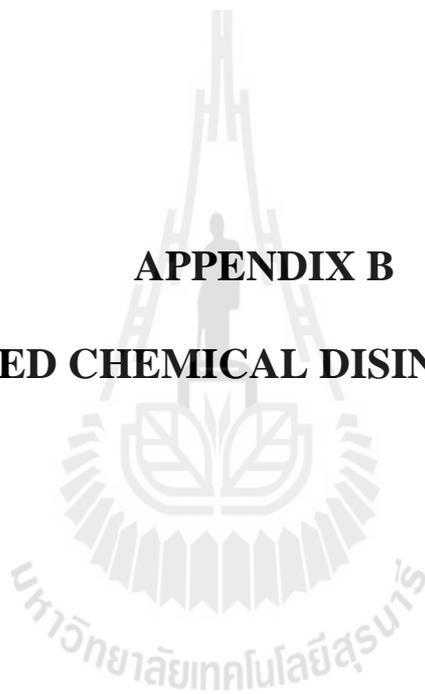
VP (Voges Proskauer) test

Ingredients (gm/L)

Alpha-naphthol 5%	50.00	gm.
Absolute ethanol	1000.00	ml.



APPENDIX B
DILUTED CHEMICAL DISINFECTANTS



APPENDIX B

DILUTED CHEMICAL DISINFECTANTS

Savlon®

Each 1000 ml. contains:

Chlorhexidine gluconate 1.5%

Cetrimide 15%

Diluted with steriled water at ratio of 1:100

Pose-cresol®

Each 5 gm. contains:

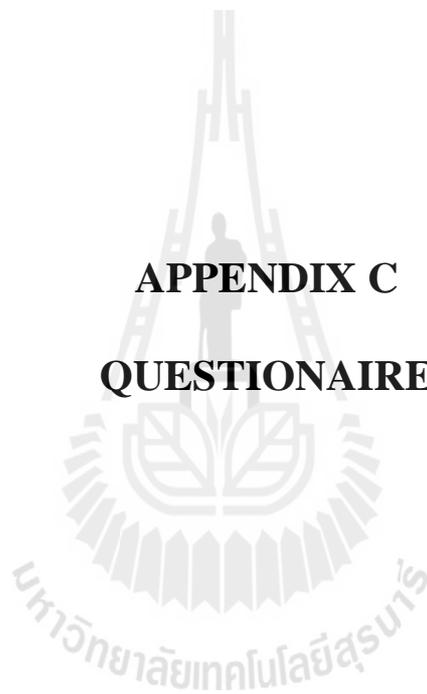
Arylphenol and halogenated alkylphenol with surfactant and other excipients
for synergistic activity

Diluted with distilled water 1,000.00 ml.

Sodium hypochlorite

Diluted with water from faucet aerator at ratio of 1:20

APPENDIX C
QUESTIONNAIRE



APPENDIX C

QUESTIONNAIRE

Questionnaire 1 for head ward:

“The epidemiology of *Pseudomonas aeruginosa* in wards at Maharat Nakhon Ratchasima Hospital.”

Instruction Please check / in follow the real data

Part 1 Personal data

Classified Register nurse Technical nurse

Age.....years

Gender male female

Ward

Part 2 Knowledges of head ward about spreading of *Pseudomonas aeruginosa* at Maharat Nakhon Ratchasima Hospital.

1. Do you know what *Pseudomonas* species is?

Yes No

2. Did *Pseudomonas aeruginosa* cause antibiotic resistant at Maharat Nakhon Ratchasima Hospital?

Yes No

3. Do *Pseudomonas* species grow in water?

Yes No

4. Did mowing and cutting trees effect on spreading of *Pseudomonas* species in the hospital?

Yes No

5. Did the sink cause spreading of antibiotic resistant gram negative bacilli bacteria?

Yes No

6. Did you think staff's sinks are clean enough?

Yes No

7. Have your staffs ever cleaned the medical equipments in the hand wash sinks?

Yes No

8. Have your staffs ever washed their hands before nursing care for each patient at the medical equipment sinks or cooking sinks?

Yes No

9. Did you know that patient's beds can spread bacteria?

Yes No

10. When the patients are discharged from the hospital, do your staffs change the bed sheets immediately?

Yes No

11. Did your staffs change the bed sheets immediately when it was found secretions or blood?

Yes No

12. When the nurse found dirt or dust contaminations, did your staffs change the bed sheets immediately?

Yes No

13. Before changing the bed sheet, did your staffs clean the mattress everytime?

Yes

No



Questionnaire 2 for nurse ward:

Questionnaire 2 “The epidemiology of *Pseudomonas aeruginosa* in wards at Maharat Nakhon Ratchasima Hospital.

Instruction Please check / in or fill in the blanks.....follow the real data

Part 1 Personal data

Classified Register nurse Technical nurse

Age.....years

Gender male female

Ward.....

Part 2 Knowledges of head ward about spreading of *Pseudomonas aeruginosa* at Maharat Nakhon Ratchasima Hospital.

1. Do you know what *Pseudomonas* species is?

Yes No

2. Did *Pseudomonas aeruginosa* cause antibiotic resistant at Maharat Nakhon Ratchasima Hospital?

Yes No

3. Do *Pseudomonas* species grow in water?

Yes No

4. Did mowing and cutting trees effect on spreading of *Pseudomonas* species in the hospital?

Yes No

5. Did the sink cause spreading of antibiotic resistant gram negative bacilli bacteria?

Yes No

6. Did you think staff's sinks are enough?

Yes No

7. Have your staffs ever cleaned the medical equipments in the hand wash sinks?

Yes No

8. Have your staffs ever washed their hands before nursing care for each patient at the medical equipment sinks or cooking sinks?

Yes No

9. Did you know that patient's beds can able to spread bacteria?

Yes No

10. When the patients are discharged from the hospital, do your staffs change the bed sheets immediately?

Yes No

11. Did your staffs change the bed sheets immediately when it was found secretions or blood?

Yes No

12. When your staffs found dirt or dust contaminations, did your staffs change the bed sheets immediately?

Yes No

13. Before changing the bed sheet, did your staffs clean the mattress everytime?

Yes No

Part 3 Standard precautions for working nurses

1. How often do you wash your hands before nursing care for each patient?

- Everytime Sometimes Never

If you sometimes wash or did not wash, please answer next question (you can answer more than 1 choice).

1.1 Cause that you sometimes wash or did not wash your hands before nursing care for each patient?

- Inconvenient
 Continuous for nursing care
 Busy
 Others.....

2. How often do you wash your hands after nursing care for each patient?

- Everytime Sometimes Never

If you sometimes wash or did not wash, please answer next question (you can answer more than 1 choice).

2.1 Cause that you sometimes wash or did not wash your hands after nursing care for each patient?

- Inconvenient
 Continuous for nursing care
 Busy
 Others.....

3. How often do you wash your hands with antiseptic?

- Everytime Sometimes Never

4. How often do you change gloves if they tear?

- Everytime Sometimes Never

5. Do you wear gloves while you are taking care of patients?

- Everytime Sometimes Never

6. Do you always use gloves when you have to touch patient's blood or secretion?

- Everytime Sometimes Never

7. Do you wash your hands after taking off your gloves?

- Everytime Sometimes Never

8. Do you change your gloves when you make a new activity?

- Everytime Sometimes Never

9. Do you wear a needle sheath by one hand?

- Every time Sometimes Never

10. Do you cut drug ampule by using cover gauze?

- Everytime Sometimes Never

11. How often do you wear mask before exposing patients?

- Everytime Sometimes Never

12. Do you always take a mask at neck when you finish your activity?

- Every time Sometimes Never

13. How often do you change mask when it is wet?

- Everytime Sometimes Never

14. Do you always wear the accessories (gloves, mask, apron, eye-glass, etc.) for protecting body before exposing patients?

- Everytime Sometimes Never

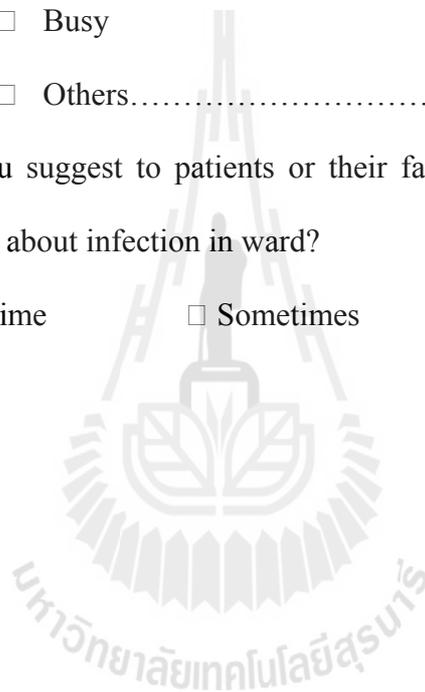
If you sometimes wear or did not wear, please answer next question (you can answer more than 1 choice).

14.1 Cause that you sometimes wear or did not wear the accessories for protecting body before exposing patients.

- Inconvenient
- Continuous for nursing care
- Busy
- Others.....

15. How often do you suggest to patients or their family members to take part in prevention themselves about infection in ward?

- Everytime
- Sometimes
- Never



แบบสอบถาม 1**(ทดลองแบบสอบถาม)****คำชี้แจง**

1. แบบสอบถามฉบับนี้เป็นการทดลองแบบสอบถามเพื่อการศึกษาค้นคว้าวิจัยซึ่งเป็นแบบสอบถามสำหรับพยาบาลหัวหน้าหอผู้ป่วย โรงพยาบาลเซนต์แมรี
2. การตอบแบบสอบถามฉบับนี้ ใช้ในทดลองแบบสอบถามเพื่อการศึกษาค้นคว้าวิจัยเท่านั้น โดยมีจุดมุ่งหมายเพื่อใช้ในการศึกษาวิจัยเรื่อง การศึกษาระบาดวิทยาของเชื้อ *Pseudomonas* species ในหอผู้ป่วย โรงพยาบาลมหาราชนครราชสีมา
3. แบบสอบถามนี้มี 2 ตอน
ตอนที่ 1 ข้อมูลส่วนบุคคล
ตอนที่ 2 ความคิดเห็นเกี่ยวกับการแพร่กระจายของเชื้อ *Pseudomonas* species ในหอผู้ป่วย
4. ข้อมูลที่ได้จากการตอบแบบสอบถามครั้งนี้จะถือเป็นความลับ ซึ่งจะไม่มีผลกระทบใด ๆ ต่อผู้ตอบแบบสอบถามไม่ว่าจะทางตรง หรือทางอ้อม

ขอขอบคุณเจ้าหน้าที่พยาบาลทุกท่านที่ให้ความร่วมมือในการตอบแบบสอบถาม

ลงชื่อ**(นางอัจฉริยา ช่างงาม)**

นักศึกษาระดับปริญญาเอก หลักสูตรสาขาวิชาชีววิทยาสิ่งแวดล้อม
สาขาวิชาชีววิทยา สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีสุรนารี

แบบสอบถาม 1

คำชี้แจง

1. แบบสอบถามฉบับนี้ เป็นแบบสอบถามสำหรับพยาบาลหัวหน้าหอผู้ป่วย โรงพยาบาลมหาราชนครราชสีมา
2. การตอบแบบสอบถามฉบับนี้ ใช้ในการศึกษาเท่านั้น โดยมีจุดมุ่งหมายเพื่อใช้ในการศึกษาวิจัยเรื่อง การศึกษาระบาดวิทยาของเชื้อ *Pseudomonas* species ในหอผู้ป่วย โรงพยาบาลมหาราชนครราชสีมา
3. แบบสอบถามนี้มี 2 ตอน
ตอนที่ 1 ข้อมูลส่วนบุคคล
ตอนที่ 2 ความคิดเห็นเกี่ยวกับการแพร่กระจายของเชื้อ *Pseudomonas* species ในหอผู้ป่วย
4. ข้อมูลที่ได้จากการตอบแบบสอบถามครั้งนี้จะถือเป็นความลับ โดยผู้ทำการศึกษาวิจัยจะนำเสนอในภาพรวมซึ่งไม่มีผลกระทบต่อผู้ตอบแบบสอบถามไม่ว่าจะทางตรง หรือทางอ้อม

ขอขอบคุณเจ้าหน้าที่พยาบาลทุกท่านที่ให้ความร่วมมือในการตอบแบบสอบถาม

ลงชื่อ

(นางอัจฉรียา ช่างงาม)

นักศึกษาระดับปริญญาเอก หลักสูตรสาขาวิชาชีววิทยาสิ่งแวดล้อม
สาขาวิชาชีววิทยา สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีสุรนารี

แบบสอบถาม 1

แบบสอบถาม 1 “การศึกษาระบาดวิทยาของเชื้อ *Pseudomonas* species ในหอผู้ป่วย โรงพยาบาล
มหาราชนครราชสีมา”

คำชี้แจง โปรดกาเครื่องหมาย ลงใน หรือเติมข้อความลงในช่องว่าง ตามความ
จริงเกี่ยวกับหอผู้ป่วยของท่าน

ตอนที่ 1 ข้อมูลส่วนบุคคล

ตำแหน่ง พยาบาลวิชาชีพ พยาบาลเทคนิค

อายุ.....ปี

เพศ ชาย หญิง

หอผู้ป่วย

ตอนที่ 2 ความรู้เกี่ยวกับการแพร่กระจายของเชื้อ *Pseudomonas* species ในหอผู้ป่วยโรงพยาบาล
มหาราชนครราชสีมา

1. ท่านรู้จักเชื้อ *Pseudomonas* species หรือไม่

รู้จัก ไม่รู้จัก

2. *Pseudomonas* species เป็นสาเหตุสำคัญของเชื้อดื้อยาปฏิชีวนะในโรงพยาบาลมหาราชนครราชสีมา ใช่หรือไม่

นครราชสีมา ใช่หรือไม่

ใช่ ไม่ใช่

3. *Pseudomonas* species สามารถเจริญในน้ำได้หรือไม่
- ได้ ไม่ได้
4. การตัดต้นไม้ตัดหญ้าสามารถทำให้เชื้อ *Pseudomonas* specie ฝังกระจายในโรงพยาบาลได้หรือไม่
- ใช่ ไม่ใช่
5. อ่างสบู่นี้เป็นสาเหตุหนึ่งในการปนเปื้อน กับการแพร่พันธุ์ของเชื้อคือยาปฏิชีวนะที่เป็นเชื้อแกรมลบ รูปร่างแท่งใช่หรือไม่
- ใช่ ไม่ใช่
6. ท่านคิดว่าจำนวนอ่างล้างมือที่มีสำหรับเจ้าหน้าที่เพียงพอหรือไม่
- เพียงพอ ไม่เพียงพอ
7. เจ้าหน้าที่ของท่านเคยมีการใช้อ่างล้างมือทำความสะอาดอุปกรณ์ทางการแพทย์ใช่หรือไม่
- ใช่ ไม่ใช่
8. เจ้าหน้าที่ของท่านเคยล้างมือก่อนให้การพยาบาลในอ่างล้างอุปกรณ์หรืออ่างล้างภาชนะสำหรับรับประทานอาหารหรือไม่
- เคย ไม่เคย
9. ท่านทราบหรือไม่ว่าเตียงผู้ป่วยสามารถแพร่กระจายเชื้อโรคได้
- ทราบ ไม่ทราบ
10. เมื่อมีการจำหน่ายผู้ป่วยออกจากเตียง เจ้าหน้าที่ของท่านเปลี่ยนผ้าปูที่นอนทันทีใช่หรือไม่
- ใช่ ไม่ใช่

11. เมื่อผ้าปูที่นอนมีการปนเปื้อนเลือด สารคัดหลั่งจากตัวผู้ป่วย เจ้าหน้าที่ของท่านเปลี่ยนผ้าปูที่นอนทันทีใช่หรือไม่

ใช่ ไม่ใช่

12. เมื่อผ้าปูที่นอนมีการปนเปื้อน ดิน ฝุ่น เจ้าหน้าที่ของท่านเปลี่ยนผ้าปูที่นอนทันทีใช่หรือไม่

ใช่ ไม่ใช่

13. ก่อนเปลี่ยนผ้าปูที่นอน เจ้าหน้าที่ของท่านทำความสะอาดเตียงผู้ป่วยทุกครั้งใช่หรือไม่

ใช่ ไม่ใช่



แบบสอบถาม 2
(ทดลองแบบสอบถาม)

คำชี้แจง

1. แบบสอบถามฉบับนี้ เป็นการทดลองแบบสอบถาม เพื่อการศึกษาค้นคว้าวิจัย ซึ่งเป็นแบบสอบถามสำหรับพยาบาลผู้ปฏิบัติงานในหอผู้ป่วย โรงพยาบาลเซนต์แมรี
2. การตอบแบบสอบถามฉบับนี้ ใช้ในการทดลองแบบสอบถามเพื่อการศึกษาค้นคว้าวิจัยเท่านั้น โดยมีจุดมุ่งหมายเพื่อใช้ในการศึกษาวิจัยเรื่อง การศึกษาระบาดวิทยาของเชื้อ *Pseudomonas species* ในหอผู้ป่วย โรงพยาบาลมหาราชนครราชสีมา
3. แบบสอบถามนี้มี 3 ตอน
 - ตอนที่ 1 ข้อมูลส่วนบุคคล
 - ตอนที่ 2 ความคิดเห็นเกี่ยวกับการแพร่กระจายของเชื้อ *Pseudomonas species* ในหอผู้ป่วย
 - ตอนที่ 3 Standard precaution สำหรับพยาบาลผู้ปฏิบัติงานในหอผู้ป่วย
4. ข้อมูลที่ได้จากการตอบแบบสอบถามครั้งนี้จะถือเป็นความลับ ซึ่งจะไม่มีผลกระทบใด ๆ ต่อผู้ตอบแบบสอบถามไม่ว่าจะทางตรง หรือทางอ้อม

ขอขอบคุณเจ้าหน้าที่พยาบาลทุกท่านที่ให้ความร่วมมือในการตอบแบบสอบถาม

ลงชื่อ

(นางอัจฉริยา ช่วงงาม)

นักศึกษาระดับปริญญาเอก หลักสูตรสาขาวิชาชีววิทยาสิ่งแวดล้อม
สาขาวิชาชีววิทยา สำนักวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีสุรนารี

แบบสอบถาม 2

คำชี้แจง

1. แบบสอบถามฉบับนี้ เป็นแบบสอบถามสำหรับพยาบาลผู้ปฏิบัติงานในหอผู้ป่วย โรงพยาบาลมหาราชนครราชสีมา
 2. การตอบแบบสอบถามฉบับนี้ ใช้ในการศึกษาเท่านั้น โดยมีจุดมุ่งหมายเพื่อใช้ในการศึกษาวิจัยเรื่อง การศึกษาระบาดวิทยาของเชื้อ *Pseudomonas* species ในหอผู้ป่วยโรงพยาบาลมหาราชนครราชสีมา
 3. แบบสอบถามนี้มี 3 ตอน
ตอนที่ 1 ข้อมูลส่วนบุคคล
ตอนที่ 2 ความคิดเห็นเกี่ยวกับการแพร่กระจายของเชื้อ *Pseudomonas* species ในหอผู้ป่วย
ตอนที่ 3 Standard precaution สำหรับพยาบาลผู้ปฏิบัติงานในหอผู้ป่วย
 4. ข้อมูลที่ได้จากการตอบแบบสอบถามครั้งนี้ จะถือเป็นความลับโดยผู้ทำการศึกษาวิจัยจะนำเสนอในภาพรวมซึ่งไม่มีผลกระทบต่อผู้ตอบแบบสอบถามไม่ว่าจะทางตรง หรือทางอ้อม
- ขอขอบคุณเจ้าหน้าที่พยาบาลทุกท่านที่ให้ความร่วมมือในการตอบแบบสอบถาม

ลงชื่อ

(นางอัจฉรียา ช่วงงาม)

นักศึกษาระดับปริญญาเอก หลักสูตรสาขาวิชาชีววิทยาสิ่งแวดล้อม
สาขาวิชาชีววิทยา สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีสุรนารี

แบบสอบถาม 2 “การศึกษาระบาดของเชื้อ *Pseudomonas* species ในหอผู้ป่วย

โรงพยาบาลมหาราชนครราชสีมา”

คำชี้แจง โปรดกาเครื่องหมาย / ลงใน หรือเติมข้อความลงในช่องว่าง ตามความ

จริงเกี่ยวกับหอผู้ป่วยของท่าน

ตอนที่ 1 ข้อมูลส่วนบุคคล

ตำแหน่ง พยาบาลวิชาชีพ พยาบาลเทคนิค

อายุ.....ปี

เพศ ชาย หญิง

หอผู้ป่วย

ตอนที่ 2 ความรู้เกี่ยวกับการแพร่กระจายของเชื้อ *Pseudomonas* species ในหอผู้ป่วยโรงพยาบาล
มหาราชนครราชสีมา

1. ท่านรู้จักเชื้อ *Pseudomonas* species หรือไม่

รู้จัก ไม่รู้จัก

2. *Pseudomonas* species เป็นสาเหตุสำคัญของเชื้อดื้อยาปฏิชีวนะในโรงพยาบาลมหาราชนครราชสีมา ใช่หรือไม่

ใช่หรือไม่

ใช่ ไม่ใช่

3. *Pseudomonas* species สามารถเจริญในน้ำได้หรือไม่

ได้ ไม่ได้

4. การตัดต้นไม้ ตัดหญ้าสามารถทำให้เชื้อ *Pseudomonas* species ฝังกระจายในโรงพยาบาลได้
ใช่หรือไม่

ใช่ ไม่ใช่

5. อ่างสบู่นั้นเป็นสาเหตุหนึ่งในการปนเปื้อน กับการแพร่พันธุ์ของเชื้อคือยาปฏิชีวนะที่เป็นเชื้อ
แกรมลบ รูปร่างแท่งใช่หรือไม่

ใช่ ไม่ใช่

6. ท่านคิดว่าจำนวนอ่างล้างมือที่มีสำหรับเจ้าหน้าที่เพียงพอหรือไม่

เพียงพอ ไม่เพียงพอ

7. เจ้าหน้าที่ของท่านเคยมีการใช้อ่างล้างมือทำความสะอาดอุปกรณ์ทางการแพทย์ใช่หรือไม่

ใช่ ไม่ใช่

8. เจ้าหน้าที่ของท่านเคยล้างมือก่อนให้การพยาบาลในอ่างล้างอุปกรณ์หรืออ่างล้างภาชนะ
สำหรับรับประทานอาหารหรือไม่

เคย ไม่เคย

9. ท่านทราบหรือไม่ว่าเตียงผู้ป่วยสามารถแพร่กระจายเชื้อโรคได้

ทราบ ไม่ทราบ

10. เมื่อมีการจำหน่ายผู้ป่วยออกจากเตียง เจ้าหน้าที่ของท่านเปลี่ยนผ้าปูที่นอนทันทีใช่หรือไม่

ใช่ ไม่ใช่

11. เมื่อผ้าปูที่นอนมีการปนเปื้อนเลือด สารคัดหลั่งจากตัวผู้ป่วย เจ้าหน้าที่ของท่านเปลี่ยนผ้าปู
ที่นอนทันทีใช่หรือไม่

ใช่ ไม่ใช่

12. เมื่อใส่ถุงมือที่มีการปนเปื้อน ดิน ฝุ่น เจ้าหน้าที่ของท่านเปลี่ยนถุงมือทันทีหรือไม่

- ใช่ ไม่ใช่

13. ก่อนเปลี่ยนถุงมือ เจ้าหน้าที่ของท่านทำความสะอาดเตียงผู้ป่วยทุกครั้งหรือไม่

- ใช่ ไม่ใช่

ตอนที่ 3 Standard precaution สำหรับพยาบาลผู้ปฏิบัติงานในหอผู้ป่วย

1. ท่านล้างมือทุกครั้งก่อนให้การพยาบาลแก่ผู้ป่วยแต่ละรายหรือไม่

- ปฏิบัติทุกครั้ง
 ปฏิบัติบางครั้ง
 ไม่ได้ปฏิบัติ

ถ้าตอบว่าปฏิบัติบางครั้ง หรือไม่ได้ปฏิบัติตามกรุณาตอบคำถามดังต่อไปนี้ (ตอบได้มากกว่า 1

ข้อ)

1.1 สาเหตุที่ทำให้ท่านล้างมือเป็นบางครั้ง หรือไม่ได้ล้างก่อนให้การพยาบาลแก่ผู้ป่วยแต่ละ

รายเพราะอะไร

- ไม่สะดวก
 ต้องให้การพยาบาลต่อเนื่อง
 ภาระงานยุ่ง
 อื่น ๆ ระบุ

2. ท่านล้างมือทุกครั้งหลังจากให้การพยาบาลแก่ผู้ป่วยแต่ละรายหรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

ถ้าตอบว่าปฏิบัติบางครั้ง หรือไม่ได้ปฏิบัติ กรุณาตอบคำถามดังต่อไปนี้ (ตอบได้มากกว่า 1

ข้อ)

2.1 สาเหตุที่ทำให้ท่านล้างมือเป็นบางครั้ง หรือไม่ได้ล้างมือ หลังจากให้การพยาบาลแก่ผู้ป่วยแต่ละรายเพราะอะไร

- ไม่สะดวก
- ต้องให้การพยาบาลต่อเนื่อง
- ภาระงานยุ่ง
- อื่น ๆ ระบุ

3. ท่านล้างมือด้วยน้ำยาฆ่าเชื้อหรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

4. ท่านเปลี่ยนถุงมือทุกครั้ง ที่ถุงมือนึกขาด หรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

5. ท่านสวมถุงมือทุกครั้ง ขณะที่ท่านให้การพยาบาลผู้ป่วย หรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

6. ท่านสวมถุงมือทุกครั้ง ที่สัมผัสกับเลือด หรือสารคัดหลั่งของผู้ป่วย หรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

7. ท่านล้างมือหลังจากที่ท่านถอดถุงมือออก หรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

8. ท่านมีการเปลี่ยนถุงมือ ก่อนที่จะให้การพยาบาลในครั้งใหม่ หรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

9. ท่านสวมปกอกเข็มด้วยมือข้างเดียว

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

10. ท่านหักหลอดยาฉีด โดยใช้ฝ้ายก๊อสรองขวดยา หรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

11. ท่านสวมหน้ากากก่อนให้การพยาบาลผู้ป่วย หรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

12. หลังจากที่ให้การพยาบาลเสร็จท่านสวมหน้ากากติดไว้ที่คอ หรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

13. ท่านเปลี่ยนหน้ากากเมื่อหน้ากากอยู่ในสภาพที่เปียก หรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

14. ท่านสวมอุปกรณ์ต่าง ๆ (ถุงมือ หน้ากาก ผ้ากันเปื้อน แว่น ฯลฯ) สำหรับป้องกันร่างกาย ก่อนให้การพยาบาลกับผู้ป่วยหรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

ถ้าท่านตอบว่า ปฏิบัติบางครั้ง หรือไม่ได้ปฏิบัติ เพราะสาเหตุใด (ตอบได้มากกว่า 1 ข้อ)

14.1 สาเหตุที่ทำให้ท่านสวมอุปกรณ์ต่าง ๆ เป็นบางครั้ง หรือไม่ได้สวมอุปกรณ์ต่าง ๆ

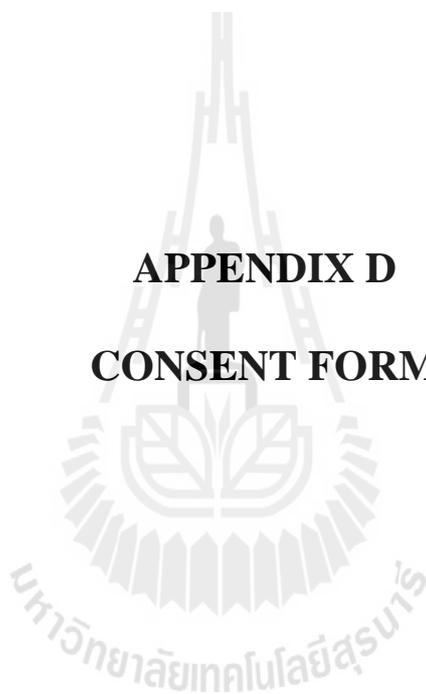
สำหรับป้องกันร่างกายก่อนทำการให้การพยาบาลกับผู้ป่วยเพราะอะไร

- ไม่สะดวก
- ต้องให้การพยาบาลต่อเนื่อง
- ภาระงานยุ่ง
- อื่น ๆ ระบุ

15. ท่านแนะนำผู้ป่วยหรือญาติผู้ป่วยในหอผู้ป่วย ถึงการมีส่วนร่วมในการป้องกันตนเอง เกี่ยวกับการติดเชื้อในโรงพยาบาล หรือไม่

- ปฏิบัติทุกครั้ง
- ปฏิบัติบางครั้ง
- ไม่ได้ปฏิบัติ

APPENDIX D
CONSENT FORM



APPENDIX D

CONSENT FORM

ข้อมูลสำหรับอาสาสมัคร

คำชี้แจง

- วัตถุประสงค์การวิจัยเพื่อเปรียบเทียบจำนวนแหล่งของเชื้อ *Pseudomonas aeruginosa* ก่อนและหลังการให้ความรู้แก่พยาบาลในหอผู้ป่วยเกี่ยวกับการป้องกันและควบคุมเชื้อ *Pseudomonas aeruginosa* ศิษยานุศิษย์ของเชื้อ *Pseudomonas* ที่มีผลโดยตรงต่อคนไข้ในหอผู้ป่วยสามัญของโรงพยาบาลมหาราช นครราชสีมา ศึกษา Multidrug-Resistant Gram-Negative Bacilli (MDR-GNB) ที่พบในแหล่งเดียวกับเชื้อ *Pseudomonas* และศึกษาประสิทธิภาพของน้ำยาฆ่าเชื้อ เจือจางพร้อมใช้ในหอผู้ป่วยของโรงพยาบาลมหาราช นครราชสีมา
- การเก็บตัวอย่างจากพลาสติกดีดแผลบนร่างกายของอาสาสมัครนี้ เป็นส่วนหนึ่งที่ใช้ในการศึกษาเท่านั้น
- การเข้าร่วมวิจัยในครั้งนี้ จะไม่ก่อให้เกิดความเจ็บปวดอันตรายต่อร่างกาย หรือจิตใจแก่อาสาสมัครทั้งทางตรง และทางอ้อมแต่อย่างใด

5. ข้อมูลที่ได้จากการเก็บตัวอย่าง จากพลาสติกดีคิเดคพลบนร่างกายของอาสาสมัครนี้ จะถือเป็นความลับ โดยผู้ทำการศึกษาวิจัยจะนำเสนอในภาพรวม ซึ่งไม่มีผลกระทบต่ออาสาสมัครไม่ว่าจะทางตรง หรือทางอ้อม

ขอขอบคุณอาสาสมัครที่เข้าร่วมการวิจัยในครั้งนี้

ลงชื่อ

(นางอัจฉริยา ช่วงงาม)

นักศึกษาระดับปริญญาเอก หลักสูตรสาขาวิชาชีววิทยาสิ่งแวดล้อม
สาขาวิชาชีววิทยา สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีสุรนารี

หนังสือแสดงความยินยอมการเข้าร่วมในงานวิจัย

ข้าพเจ้า นาย/นาง/นางสาว.....ขอให้ความยินยอมของตนเองที่จะเกี่ยวข้องในการวิจัยเรื่อง การศึกษาระบาดวิทยาของเชื้อ *Pseudomonas* species ในหอผู้ป่วยโรงพยาบาลมหาราชนครราชสีมา ซึ่งผู้วิจัยได้แก่ นางอัจฉริยา ช่วงงาม ได้อธิบายต่อข้าพเจ้าเกี่ยวกับการวิจัยครั้งนี้แล้ว ผู้วิจัยมีความยินดีที่จะตอบคำถาม ที่เกี่ยวข้องกับการเข้าร่วมในงานวิจัยตลอดระยะเวลาที่เข้าร่วมการวิจัยในครั้งนี้ และรับรองว่าข้อมูลเฉพาะที่เกี่ยวกับตัวข้าพเจ้าจะถือเป็นความลับ โดยผู้วิจัยจะนำเสนอในภาพรวม ซึ่งไม่มีผลกระทบใด ๆ ต่อข้าพเจ้าไม่ว่าจะทางตรงหรือทางอ้อม และการเข้าร่วมวิจัยในครั้งนี้ จะไม่ก่อให้เกิดความเจ็บปวด อันตรายต่อร่างกายหรือจิตใจแก่ข้าพเจ้าทั้งทางตรงและทางอ้อมตลอดการวิจัย และรับรองว่า หากเกิดอันตรายใด ๆ จากการวิจัยดังกล่าว ผู้ยินยอมจะได้รับการรักษาอย่างเต็มที่ ข้าพเจ้ายินยอมเข้าร่วมการวิจัยโดยสมัครใจ และสามารถถอนตัวจากการวิจัยนี้เมื่อใดก็ได้ โดยไม่มีผลกระทบต่อการรักษาพยาบาลที่ข้าพเจ้าจะได้รับ และในกรณีที่เกิดข้อข้องใจ หรือมีปัญหาที่ข้าพเจ้าต้องการปรึกษากับผู้วิจัย ข้าพเจ้าสามารถติดต่อกับผู้วิจัยคือ นางอัจฉริยา ช่วงงาม ได้ที่สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีสุรนารี สาขาชีววิทยา โทรศัพท์ 0-4422-4633 โทรสาร 0-4422-4633

ลงนาม.....ผู้ยินยอม

ลงนาม.....สามี / ภรรยา / ผู้ปกครองของผู้ยินยอม

ลงนาม.....ผู้วิจัย

ลงนาม.....พยาน

ลงนาม.....พยาน

วันที่...../...../.....



CURRICULUM VITAE

NAME MRS. ATCHAREEYA CHOUNGNGAM

DATE OF BIRTH 16 October, 1977

PLACE OF BIRTH UDONTANEE, THAILAND

INSTITUTIONS ATTENDED

Bachelor of Nursing Science, 1999

Boromarajonani College of Nursing Nakhonratchasima

Master of Education, 2004

Maharakham University

Ph.D. of Science in School of Biology, 2009

Suranaree University of Technology

OCCUPATION Nurse of Maharat Nakhon Ratchasima Hospital.