RISK FACTORS OF CEFOPERAZONE/SULBACTAM RESISTANT *ACINETOBACTER BAUMANNII* NOSOCOMIAL INFECTION AT RAJAVITHI HOSPITAL

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ABSTRACT

Acinetobacter baumannii is an microorganism that probably developed into various drug-resistant pathogens, moreover, it can increase the mortality rate. A hospital-based case-control study was used to assess factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. Patients admitted in Rajavithi Hospital, Bangkok, Thailand from January 1 to November 30, 2007, were used for the study. 148 patients with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection were assigned as case group and 295 with cefoperazone/sulbactam-susceptible *A. baumannii* nosocomial infection were used as control group. The average age of the study population was 62.56±19.6 years among cases and 58.31±20.3 years among controls. The mean length of hospital stay among cases was 7.8±7.28 weeks and 6.89±7.35 weeks among controls. The type of specimens among case was mostly sputum (62.8%). The pathogen isolated with *A. baumannii* among cases was mostly *E. coli* (33.1%). The incidence of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection was 6.2/1,000 patients, the fatality rate was 62.2 % for cases and 42.2 % for controls. The results from multiple logistics showed significant associations between certain factors and cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. There were malignancy (OR= 3.00, 95%CI= 1.48-6.10), chronic renal failure (OR= 2.90, 95%CI= 1.39-6.05), retained nasogastric intubation (OR= 2.13, 95%CI= 1.13-4.01), and retained nasogastric intubation > 1 week (OR= 2.38, 95%CI= 1.21-4.70).

The incidence of cefoperazone/sulbactam-resistant *A. baumannii* infection corresponds to the incidence of *A. baumannii* infection. Risk factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection were malignancy, chronic renal failure and retained nasogastric intubation.

KEY WORDS: RISK FACTORS / CEFOPERAZONE/SULBACTAM RESISTANT */ACINETOBACTER BAUMANNII* / NOSOCOMIAL INFECTION

85 pages

ปัจจัยเสี่ยงของการติดเชื้อ *Acinetobacter baumannii* ที่ดื้อต่อยาเซฟโฟเพอราโซล/ซัลแบกแทมในโรงพยาบาลราชวิถี

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บทคัดย่อ

Acinetobacter baumannii เป็นเชื้อฉวยโอกาสที่ก่อโรคในโรงพยาบาล และเพิ่มอัตราตายของผู้ป่วยให้สูงขึ้น การศึกษานี้เป็นการศึกษาแบบย้อนหลัง เพื่อศึกษาปัจจัยที่มีความสัมพันธ์กับการติดเชื้อ *A. baumannii* ที่ดื้อต่อยา เซฟโฟเพอราโซล/ซัลแบกแทม โดยทำการศึกษาวิจัยในผู้ป่วยที่เข้านอนรักษาในโรงพยาบาลราชวิถี ระหว่างวันที่ 1 มกราคม 2550 ถึง 30 พฤศจิกายน 2550 โดยใช้แบบเก็บข้อมูลที่ผู้วิจัยสร้างขึ้น กลุ่มตัวอย่างที่ศึกษาคือ ผู้ป่วยที่ติดเชื้อ *A. baumannii* ที่ดื้อต่อยาเซฟโฟเพอราโซล/ซัลแบกแทม จำนวน 148 ราย และกลุ่มเปรียบเทียบคือ ผู้ป่วยที่ติดเชื้อ *A. baumannii* ที่ไม่ดื้อยา เซฟโฟเพอราโซล/ซัลแบกแทม จำนวน 295 ราย ผลการศึกษาพบว่า กลุ่มศึกษามีอายุเฉลี่ย 62.56±19.6 ปี และในกลุ่มเปรียบเทียบ 58.31±20.3 ปี ระยะเวลาการนอนโรงพยาบาลเฉลี่ยในกลุ่มศึกษา 7.8±7.28 สัปดาห์ และในกลุ่มเปรียบเทียบ 6.89±7.35 สัปดาห์ สิ่งส่งตรวจที่พบเชื้อ *A. baumannii* ในกลุ่มศึกษา มากที่สุดคือ เสมหะ 62.8 % เชื้อจุลชีพที่สามารถตรวจพบพร้อมกับ *A. baumannii* ในกลุ่มศึกษามากที่สุดคือ *E. coli* 33.1% อุบัติการการติดเชื้อ *A. baumannii* ที่ดื้อต่อยา เซฟโฟเพอราโซล/ซัลแบกแทม คือ 6.2 ต่อผู้ป่วย 1,000 คน อัตราตายเฉพาะสาเหตุในกลุ่มศึกษา คือ ร้อยละ 62.2 ในกลุ่มเปรียบเทียบ คือ ร้อยละ 42.2 ผลการวิเคราะห์ตัวแปรเชิงซ้อนพบว่า ปัจจัยที่มีความสัมพันธ์กับการติดเชื้อ *A. baumannii* ที่ดื้อต่อยาเซฟโฟเพอราโซล/ซัลแบกแทม ได้แก่ โรคมะเร็ง (OR= 3.00, 95%CI= 1.48-6.10), โรคไตวายเรื้อรัง (OR= 2.90, 95%CI= 1.39-6.05), การใส่สายยางให้อาหารทางจมูกสู่กระเพาะอาหารนานกว่า 1 สัปดาห์ (OR= 2.38, 95%CI= 1..21-4.70)

ผลการศึกษานี้แสดงให้เห็นว่า อัตราอุบัติการณ์ของการติดเชื้อ *A. baumannii* ที่ดื้อต่อยาเซฟโฟเพอราโซล/ซัลแบกแทม สอดคล้องไปกับการติดเชื้อ *A. baumannii* ปัจจัยที่มีผลกับการติดเชื้อ *A. baumannii* ที่ดื้อต่อยา เซฟโฟเพอราโซล/ซัลแบกแทม คือ โรคมะเร็ง, โรคไตวายเรื้อรัง และการใส่สายยางให้อาหารทางจมูกสู่กระเพาะอาหาร

85 หน้า

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CHAPTER I
INTRODUCTION

1. Rationale and Background

Acinetobacter baumannii is an encapsuled gram negative coccobacilli bacteria that is generally non motile, it is an opportunistic pathogen found in soil and water(1). *A. baumannii* is able to survive on various surfaces in hospital that are abiotic, wet or dry and has the ability to avoid desiccation for more than 30 days on dry surfaces (2). *A. baumannii* has ability to live on a variety of medical equipment such as surgical drains and catheters (3), also it can spread around hospital by health-care workers (4). It has became the major cause of nosocomial infection for over the past two decades (5). Formerly, *A. baumannii* was susceptible to common antimicrobiotic, but now it has developed to be multidrug-resistance organism(6). In 1999, it was reported from 15 hospitals in Blooklyn reported a total of 419 isolates of *A. baumannii* were found, out of which 53% were resistant to meropenem and/or imipenem, and 12% were resistant to all common antibiotic (7), A numerous cases are reported worldwide being documented show increasing antimicrobial resistance among *A. baumannii* clonical isolates. The problem of antimicrobial-resistance *A. baumannii* has become more intensive in ICU patients not only in Europe but also in Asian and south American (8).

In Thailand, a 1998 study showed that 4% of *Acinetobacter* spp. isolates were resistant to imipenem (9), the incidence of imipenem-resistant *Acinetobacter* spp. has dramatically increased to over 50% (10). The impact of drug-resistant *A. baumannii* nosocomial infection is a major impact on the mortality rate, Kwon KT. *et al* found higher at the patients. When compared with patients who had non imipenem-susceptible *A. baumannii*, the mortality rate were 57.5% and 27.5%, respectively (11). Lee NY. *et al* identified patients with multidrug-resistant *A. baumannii* had a higher mortality rate than patients with non multidrug-resistant

A. baumannii (12). In Thailand, a study at Songklanagarind Hospital revealed that the mortality rate in the patients with imipenem-resistant *A. baumannii* compared to patients with imipenem-susceptible *A. baumannii* were 33.8% and 24.1%, respectively (13). Drug-resistance to *A. baumannii* caused to increase the length of hospital stay, in 2005, the Tiwan study found that the difference in mean length of hospital stay in patients who had multidrug-resistance to *A. baumannii* nosocomial infection compared to patients who had non multidrug-resistance *A. baumannii* nosocomial infection (54.2 days V.S. 34.1 days)(12). The some on a report from Thailand the length of hospital stay was 4.9 weeks in multidrug-resistant *A. baumannii* and 1.8 weeks in non multidrug-resistant *A. baumannii* infection (14). This caused the higher cost of treatment, cost in multidrug-resistant group were 9,349 dollars but in non multidrug-resistant group only 4,885 dollars (12).

Drug-resistant *A. baumannii* is problematic nosocomial pathogen. There were many reports about drug-resistant *A. baumannii* nosocomial infections, especially efficacy of drug which can treat drug-resistant *A. baumannii* . In the year 1997, Manikal VM. *et al* presented a report from 11 hospitals in Brooklyn, New York, the susceptible rates were as the following: polymycine, 99%; amikacin, 87%; ampicillin/sulbactam, 47%; ceftazidime, 25%; and ciprofloxacin 23% (15). The study about nosocomial bacteremia nosocomial by BMA Medical College and Vajira Hospital compared between 2000-2002 and 2003-2005, they found that most of gram negative pathogens were all sensitivity to ciprofloxacin except *A. baumannii*, the rate of susceptible was only 61 % and most gram negative pathogens were 80-100 % sensitivity to cabapenem except *A. baumannii* was only 67-86 % (16). Recent studies about of efficiency of cefoperazone/sulbactam by the division of infectious disease, (Sao Paulo University Hospital, Brazil) reports the emergence of infections involving multidrug-resistant *A. baumannii* clinical was isolated. Sulbactam has direct antimicrobial activity against *A. baumannii* species. Accordingly, co-administration of sulbactam with cefoperazone offers the potential of effective empirical therapy against *Acinetobacter* spp. institutions in which they are susceptible (17). In 1996, China had to evaluated the efficacy and safety of cefoperazone/sulbactam compared with imipenem in the treatment of respiratory tract infections. The overall clinical efficacy rates of cefoperazone/sulbactam and

imipenem were 93.5% and 93.3%, respectively. The susceptibility rate of bacteria isolated to two drugs were 95.9% and 93.9%, respectively(18). Choi JY. *et al* had compared the outcome between *Acinetobacter* bacteriamia patients treated by cefoperazone/sulbactam and imipenem. The mortality rate were same two group (19).

Data from National Antimicobial Resistance Surveilance Center Thailand (2003 to 2007) showed that the in vitro activities of common antibiotics against *A. baumannii*. The resistant rate of imipenem decreased from 29% to 58%, and for sulperazone from 8% to 15% (10). In Rajavithi Hospital, Medical School, Rangsit University they used many antibiotics for treating infections from *A. bamanni*, but mainly included imipenem and cefoperazone/sulbactam It has been observed that many patients resistance to imipenem but cefoperazone/sulbactam reported only few resistant *A. baumannii* bacteremia. This is an interesting cause if we are able the risk factors for *A. baumannii* infection that resistant cefoperazone/sulbactam-resistance. It is both advantageous to patients and hospital. This study will help us to prevent or slow down the rate of cefoperazone/sulbactam-resistance *A. baumannii* nosocomail infection overal decrease the cost of treatment. In current, Rajavithi Hospital must should purchasing antibiotic that was resistant to microbe which can treat infecting *A. baumanni* that mostly antibiotic in current *A. baumanni* will resist its nearly all and antibiotic was found to be as useful for treating patients with cefoperazone/sulbactam is cheap among the antimicrobials for treatment of *A. baumannii*, imipenem (tienem) cost 794 baht per vial, meropenem(meronem) cost 1,419 baht per vial, cefoperazone/sulbactam (sulperazone) cost 395 baht per vial. So, cefoperazone/sulbactam is more cost effective treatment among effective antimicrobials. It can not only control nosocomial infection but also reduce incidence and fatality rate of drug-resistant *A. baumannii* infection.

2. General objective

To study the factors associated with sulperazone-resistant *Acinetobacter baumannii* nosocomial infections in patients admitted at Rajavithi Hospital, Medical School, Rangsit University during the period of January 1 to November 30, 2007.

3. Specific objectives

1. To study the incidence of cefoperazone/sulbactam-resistant *A. baumannii* infection among the patients admitted at Rajavithi Hospital during the study period.

2. To describe the general characteristics of the patients infected with sulperazone-resistant *A. baumannii*.

3. To study case fatality rate of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection in patients admitted at Rajavithi Hospital during the study period.

4. To determine the association between cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections and the followings:

Patient factors - age, gender and co-morbidity;

Clinical and treatment factors - hospital days prior to infection, mechanical ventilation, immunosuppressive treatment, invasive procedures, surgery and antimicrobial agents, co-infection of other bacteria.

Environment factor-ward admission.

4. Hypothesis

Patient, treatment, environment factors and co-isolated microorganisms are associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections in the patients admitted at Rajavithi Hospital.

5. Definition of Variables

Nosocomial infection refers to an infection that is caused by staying in hospital that was not present or incubating at the time of admission to the hospital. An infection is considered nosocomial if it occurs 48 houres or more after admission. Nosocomial infctions diagnosed by using the criteria defined by the CDC.

Cefoperazone/sulbactam-resistant *A. baumannii* refered to *A. baumannii* that was resistant to cefoperazone 75 µg/sulbactam 30 µg by disk diffusion method in accordance with guidelines of Clinical and Laboratory Standards Institute (CLSI, 2007). Inhibition zone diameter of less than or equal to 15 mm is considered as resistant.

Risk factors refers to factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection such as, co-morbidity, duration of admission prior sulperazone resistant *A. baumannii* nosocomial infection, immunosuppressive treatment, mechanical ventilation, surgery, invasive procedures and antimicrobial drug use while admission at Rajavithi Hospital and before the first report of sulperazone sensitivity test .

Co-morbidity refers to the underlying diseases or chronic diseases had before or at the time of admission (e.g., diabetes mellitus, cancer, renal failure, HIV, heart diseas, neurological disease, lung disease, anemia).

Duration of admission prior to *A. baumannii* infection refers to period time of admission before the first report of cefoperazone/sulbactam sensitivity test and cut off by mean of time at risk classified into; time risk ≤ 7 days and time at risk > 7 days.

Immunosuppressive treatment refers to steroid use, chemotherapy and radiotherapy classified into; yes, no.

Surgery refers to receiving major surgery before the isolation of *A. baumannii* infection classified into; yes, no.

Invasive procedures refers to duration of admission using invasive treatment; retained urinary catheter, mechanical ventilation, central venous line, nasogastric intubation before until the first report of cefoperazone/sulbactam sensitivity test classified into; non use invasive procedure, use ≤ 7 days and use > 7 days.

Co-infection bacteria refers to co-infected organisms other *A. baumannii* (e.g., *Pseudomonas aeruginosa, Escherichia coli, Proteus* spp., *Enterobacter* spp., *Staphylococcus aureus*, *Klebsiella* spp.) before or at the same time had first report of cefoperazone/sulbactam sensitivity *A. baumannii* test classified into; yes, no.

Antimicrobial agents refers to the antimicrobial drugs for used before and still used until the first report of cefoperazone/sulbactam sensitivity test classified into; non use antimicrobial, use ≤ 7 days and use > 7 days.

CHAPTER II
LITERATURE REVIEW

1. Definition of nosocomial infection (20).

Nosocomial infections, usually becomes evident 48 hours or more after admission. However, because the incubation periods vary with at different of pathogens and to some extent with the patient's underlying conditions, each infection must be assessed individually for evidence that links it to the hospitalization. There are several other important principles upon which nosocomial infection definitions are based.

First, the information used to determine the presence and classification of an infection should be a combination of clinical findings, at laboratory result and other tests. Clinical evidence is derived from direct observation of the infection site or review of other pertinent sources of data, such as the patient's chart. Laboratory evidences include the results of cultures, antigen or antibody detection tests, or microscopic visualization. Supportive data are derived from other diagnostic test, such as x-ray, ultrasound, computed tomography (CT) scan, magnetic resonance imaging (MRI), radiolabel scan, endoscopic procedure, biopsy, or needle aspiration. For infections whose clinical manifestations in neonates and infants are different from those in adults so, specific criteria are applied.

Second, a physician's or surgeon's diagnosis of infection derived from direct observation during a surgical operation, endoscopic examination, or other diagnostic tests or from clinical judgment is an acceptable criterion for an infection, unless there is compelling evidence to the contrary (e.g., information written in the wrong patient's record, presumptive diagnosis that was not substantiated by subsequent studies). For certain sites of infection, however, a physician's clinical diagnosis in the absence of supportive data must be accompanied by initiation of appropriate antimicrobial therapy to satisfy the criterion.

There are two special situations in which an infection is considered nosocomial:

1. infections that are acquired in the hospital and the infection after but does not become evidence until hospital discharge but related to that admission.

2. infection in a neonate that results from passage through the birth canal.

There are two special situations in which an infection is not considered nosocomial:

1. infection that is associated with a complication or extension of infection already present on admission, unless a change in pathogen or symptoms strongly suggests the acquisition of a new infection, and

2. in an infant, an infection that is known or proved to have been acquired transplacentally (e.g., toxoplasmosis, rubella, cytomegalovirus, or syphilis) and becomes evident at or before 48 hours after birth. There are two conditions that are not infections:

colonization, which is the presence of microorganisms(on skin, mucous membranes, in open wounds, or in excretions or secretions) that are not causing adverse clinical signs or symptoms.

inflammation, which is a condition that results from tissue response to injury or stimulation by noninfectious agents, such as chemical.

2 *Acinetobacter baumannii*

2.1 Cell structure and metabolism

A. bamannii is an opportunistic pathogen found in soil and water. *A. baumannii* is an encapsulated gram negative coccobacilli bacteria that is generally non motile(21). Features on the outer cell membrane include porins and efflux channels which contribute to antibiotic resistance. In general, porins are protein channels that allow the transport of molecules across the membrane and are also sites of attachment for antibiotics. However, *A. baumannii* has fewer and smaller porins than other Gram-negative bacteria, thereby decreasing cell permeability and increasing antibiotic resistance. Interestingly, less than 5% of molecules are permeable to the cell membrane, which is less than that found in *Escherichia coli* (22).

Efflux pumps located in the cell membrane are used to pump chemicals and antibiotics out of the cell. Efflux pumps in *A. baumannii* include resistance to tetracycline called Tet (A) and Tet (B), part of the major facilitator superfamily (MFS) and functions in the exchange of protons and tetracycline(22). There is also resistance-nodulation-cell division (RND) efflux pumps found in the strain *A. baumannii* BM4454 that is encoded by the gene adeB which provides aminoglycoside resistance(23).

The cell wall on *A. baumannii* is not static, but rather changes in response to environmental conditions. In one study it was discovered that when placed in dry conditions there was an increase in the thickness of the cell wall, caused by a change in distance of the outer membrane and plasma membrane. Also, there was a change in the shape of *A. baumannii* from rod shaped to cocci through a decrease in cell division(24).

A. baumannii is a non fermentative glucose, oxidase negative, aerobic bacteria. Antibiotic resistant strains of *A. baumannii* produce beta-lactamases which can prevent antibiotic function by hydrolyzing penicillins, cephalosporins, and carbapenems. One of the first beta-lactamases, called ARI-1, but later changed to OXA-23 was collected in 1985 in Scotland, which hydrolyzed carbapenem. Other OXA-type beta-lacamases include OXA-24 and OXA-58(21).

2.2 Ecology

A. baumannii is able to survive on various surfaces in the hospital that are abiotic, wet or dry. *A. baumannii* has the ability to avoid desiccation for more than 30 days on dry surfaces, although survival rate depends on where the specific strain originally came from (e.g. wet or dry conditions) (24). One strain of *A. baumannii*, 1960 is capable of forming biofilm on glass and plastic surfaces via pili formation. The production of biofilm may explain how *A. baumanni* can survive in different types of conditions in the hospitals, including static conditions such as bed sheets and furniture, while also capable of living in harsh conditions such as catheters and respiratory tubes. *A. baumannii* also produces exopolysaccharides which strengthens the biofilm(25).

2.3 Pathology

A. baumannii causes 2-10% of all gram negative infections in the U.S. and Europe, poses little risk to healthy individuals, but generally causes infections to those with weakened immune systems(26). Specifically, the intensive care unit (ICU) in hospitals houses patients with susceptible immune systems and is normally equipped with ventilators and invasive equipment such as catheters, that are factors that can contribute to *A. baumannii* infections such as pneumonia, meningitis, septicemia, and urinary and respiratory tract infections. In one study, it was shown that *A. baumannii* could cause apoptosis or cell death to human laryngeal epithelial cells via an outer membrane protein (OMP 38). OMP38 releases cytochrome c and an apoptosis-inducing factor which enters the epithelial cell nucleus and causes the degradation of DNA (180bp)(1). The ability of *A. baumannii* to cause untreatable infections is due to a variety of antibiotic resistance genes and cell surface structures that prevent the influx of antibiotics (see cell surface structure and metabolism). Since *A. buamannii* is commonly found in hospitals which is an environment where "antibiotics are frequently used, possessing integrons with multiple resistance determinants confers a strong selective advantage"(27).

2.4 Epidemiology of *A. baumannii* infectiobn

Historically, *A. baumannii* has been a pathogen in hot and humid climates, where it has been a major cause of infections, particularly in intensive care units and sometimes a cause of community acquired pneumonia. *A. baumannii* was cited as the cause of 17% of cases of ventilator-associated pneumonias in a Guatemalan ICU second only to pseudomonas, which caused 19% of cases-years before becoming a concern in ICUs in the United States(28). Over the past two decades, *A. baumannii* infections have become an increasingly common nosocomial problem in temperate climates.

2.4.1 Epidemiology of *A. baumannii* infectiobn associated with health Care

Most information about health care associated *A. baumannii* infections is based on the outbreak investigations(29). Infections with *A. baumannii* trend to occur in debilitated patients, mostly in ICU. Residents of long-term care

facilities, particularly facilities caring for ventilator-dependent patients, are at increased risk. In addition to a stay in the ICU, risk factors for colonization and infection are recent surgery, central vascular catheterization, tracheostomy, mechanical ventilation, enteral feedings, and the treatment with third-generation cephalosporin, fluoroquinolone, or carbapenem antibiotics(15, 30).

A. baumannii outbreaks have been traced to common-source contaminations, particularly contaminated respiratory therapy and ventilator equipment, to cross-infection by the hands of health care workers who have cared for colonized or in-fected patients or touched contaminated fomites, and to the occasional health care workers who carries an epidemic strain(29, 31, 32). Once introduced into a hospital, *Acinetobacter* spp. often has an epidemiologic pattern of serial or overlapping outbreaks caused by various multidrug-resistant strains, with subsequent endemicity of multiple strains and a single endemic strain predominating at any one time. Prolonged colonization for up to 42 months and affecting 17% of patients in one study may contribute to the endemicity of *A. baumannii* after an outbreak(33).

Dramatic multihospital outbreaks have been described in Brooklyn, Chicago, northwestern Indiana, Detroit, and cities in Europe, South.America, Africa, Asia, and the Middle East(15, 34-36). A single-strain outbreak monoclonal, as identified by molecular typing of carbapenemase producing (OXA-40) *Acinetobacter* was described recently in Chicago and neighboring northwestern Indiana(36). Since 2005, at least five hospitals, three long-term care facilities, and more than 200 patients have been affected by this outbreak. In a French multicity, monoclonal outbreak of multidrug-resistant *A. baumannii*, 290 isolates were collected in 53 hospitals from April 2003 to June 2004. The epidemic strain harbored an extended spectrum ß-lactamase known as VEB-1. Most infected patients were in ICUs, medical wards, or long-term care facilities(34).

The occurrence of monoclonal outbreaks in multiple hospitals suggests interinstitutional spread, presumably by movement of patients or personnel, or exposure to common source contamination of food or equipment. Such outbreaks highlight the importance of ongoing surveillance, interfacility communication, and measures to prevent the introduction of *Acinetobacter* spp. into, and the spread from, nursing homes.

2.4.2 Epidemiology of *A. baumannii* infection associated with Seasonal Variation

Since 1974, the CDC has noted higher rates of nosocomial *Acinetobacter* infections in the summer than in other seasons(5, 37). Mcdonald evaluated 3447 *Acinetobacter* infections in adults and children in ICUs that were reported to the CDC between 1987 and 1996; infection rates were approximately 50% higher from July to October than at other times of the year. Possible explanations include warmer, more humid ambient air, which favors the growth of *Acinetobacter* in its natural habitats, and potentially preventable environmental contaminants, such as condensate from air-conditioning units, which has been implicated as a cause of epidemic *Acinetobacter* infections(5).

3. Antibiotic Resistance

Drug resistance is the reduction in the effectiveness of a drug in curing a disease or improving a patient's symptoms. When the drug is not intended to kill or inhibit a pathogen, then the term is equivalent to dosage failure or drug tolerance. More commonly, the term is used in the context of diseases caused by pathogens. Pathogens are said to be drug-resistant when drugs meant to neutralize them have reduced effect. When an organism is resistant to more than one drug, it is said to be multidrug resistant.

Antibiotic resistance is the ability of a microorganism to withstand the effects of antibiotics. It is a specific type of drug resistance. Antibiotic resistance evolves naturally via natural selection acting upon random mutation, but it can also be engineered by applying an evolutionary stress on a population. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange. If a bacterium carries several resistance genes, it is called multidrug-resistant or, informally, a superbug. The term antimicrobial resistance is sometimes use to explicitly encompass organisms other than bacteria.

Antibiotic resistance can also be introduced artificially into a microorganism through transformation protocols. This can aid in implanting artificial

genes into the microorganism. If the resistance gene is linked with the gene to be implanted, the antibiotic can be used to kill off organisms that lack the new gene.

Antibiotic resistance can be a result of horizontal gene transfer and also of unlinked point mutations in the pathogen genome and a rate of about 1 in 108 per chromosomal replication. The antibiotic action against the pathogen can be seen as an environmental pressure; those bacteria which have a mutation allowing them to survive and to reproduce. They will then pass this trait to their offspring, which will result in a fully resistant colony.

3.1 Mechanisms of resistance

Resistance mechanisms that are expressed frequently in nosocomial strains of *Acinetobacter* include β-lactamases, alterations in cell-wall channels (porins), and efflux pumps. *A. baumannii* can become resistant to quinolones through mutations in the genes gyrA and parC and can become resistant to aminoglycosides by expressing aminoglycoside-modifying enzymes(38). AmpC β-lactamases are chromosomally encoded cephalosporinases intrinsic to all *A. baumannii*. Usually, such β-lactamases have a low level of expression that does not cause clinically appreciable resistance; however, the addition of a promoter insertion sequence, ISAba1, next to the ampC gene increases β-lactamase production, causing treatment limiting resistance to cephalosporins(39). Although porin channels in *A. baumannii* are poorly characterized, it is known that reduced expression or mutations of bacterial porin proteins can hinder passage of β-lactam antibiotics into the periplasmic space, leading to antibiotic resistance.

Overexpression of bacterial efflux pumps can decrease the concentration of β-lactam antibiotics in the periplasmic space. To cause clinical resistance in *Acinetobacter*, efflux pumps usually act in association with overexpression of AmpC β-lactamases or carbapenemases. In addition to removing β-lactam antibiotics, efflux pumps can actively expel quinolones, tetracyclines, chloramphenicol, disinfectants, and tigecycline(40).

Most clinically troubling are *Acinetobacter* that acquired β-lactamases, including serine and metallo-β-lactamases, which confer resistance to carbapenems. *Acinetobacter* has ten acquired extended-spectrum β-lactamase carriage but is not as

widespread as in *Klebsiella pneumoniae* or *Escherichia coli*(41). A recent report described a "resistance island" containing 45 resistance genes within the acinetobacter genome(26). Resistance islands comprise one or more virulence genes located in a mosaic distribution within a large genomic region(42).

Currently, the term "multidrug resistance" in reference to *Acinetobacter* does not have a standard definition. It is sometimes used to denote resistance to three or more classes of drugs that would otherwise serve as treatments for acinetobacter infections (e.g., quinolones, cephalosporins, and carbapenems). The term "panresistance" has been used to describe strains of acinetobacter that are resistant to all standard antimicrobial agents tested (except colistin)(43).

3.2 Epidemiology of drug-resistance *A. baumannii*

A. baumannii was susceptible to common antibiotics, but has now developed in multidrug-resistant bacteria, capable of acquiring resistance genes. One of the first antibiotic resistant strains of *A. baumannii* called carbapenem-resistant *A. baumanni* was isolated in 1991(44). *A. baumannii* generally affects patients with low immune systems, which has caused nosocomial infections and major concerns in hospitals given the ability of *A. baumannii* to live on a variety of hospital surfaces such as surgical drains and catheters(45). Recently, there has been a growing number of blood stream infections caused by multidrug resistant *A. baumanni* among service members of the Iraq and Afghanistan military operations: Operation Iraqi Freedom and Operation Enduring Freedom, respectively(27). *Acinetobacter* can cause many type of infection by pneumonia, urinary tract infections, and septicaemia(21).

Tong MJ. reported on 63 soldiers with soft-tissue *Acinetobacter* infections(46, 47). Most recently, *A. baumannii* infections have been reported among U.S. military personnel injured in the Middle East(27, 48-51). From January 2002 to August 2004, 85 bloodstream infections with *A.baumannii* were identified in soldiers in two military referral hospitals; the soldiers had been injured during Operation Enduring Freedom in Afghanistan and Operation Iraqi Freedom in the Iraq Kuwait region. A total of 35% of the isolates were susceptible only to imipenem, and 4% showed resistance to all standard drugs(48). According to another report, among 142 acinetobacter isolates recovered from October 2003 to November 2005, strains from

deployed personnel showed a lower rate of susceptibility to imipenem than isolates from nondeployed personnel (63% vs. 87%, P<0.01)(49).

Several studies have demonstrated that patterns of antibiotic usage had greatly affected the number of resistant organisms which develop. Overuse of broad-spectrum antibiotics, such as second- and third generation cephalosporins, greatly hastens the development of methicillin resistance. Other factors that contributing towards resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, the impregnation of household items and children's toys with low levels of antibiotics, and the use of antibiotics as livestock food additives for growth promotion.

3.3 Cause of antibiotic resistance (52)

In bacterial populations mutations are constantly arising due to the errors that occur during replication. If there is any selective advantage for a particular mutation (e.g. antibiotic resistance), the mutant will quickly become the major clone of the population due to the rapid growth rate of that bacteria. In addition, since bacteria are haploid organisms, even mutations that might normally be recessive will be expressed. Thus, mutations in bacterial populations can pose a problem in the treatment of bacterial infections. Not only having mutations a problem, also bacteria have the mechanisms to transfer these same to other bacteria. Thus, a mutation arising in one cell can be passed on to other cells.

Gene transfer in bacteria is unidirectional from a donor cell to a recipient cell and the donor usually gives only a small part of its DNA to the recipient. Thus, complete zygotes are not formed; rather, partial zygotes (merozygotes) are formed. Bacterial genes are usually transferred to members of the same species but occasionally bacterial transfer to the other species.

3.3.1 Gene transfer mechanisms in bacteria(52)

Transformation

Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell. Certain bacteria (e.g. *Bacillus, Haemophilus, Neisseria, Pneumococcus*) can take up DNA from the environment and the DNA that is taken up can be incorporated into the recipient's chromosome.

Factors affecting transformation

DNA size state, double stranded DNA of at least 5 X 105 daltons works best. Thus, transformation is sensitive to nucleases in the environment.

Competence of the recipient, some bacteria are able to take up DNA naturally. However, these bacteria only take up DNA a particular time in their growth cycle when they produce a specific protein called a competence factor. At this stage the bacteria are said to be competent. Other bacteria are not able to take up DNA naturally. However, in these bacteria competence can be induced in vitro by treatment with chemicals.

Steps in transformation

Uptake of DNA, uptake of DNA by gram positive and gram negative bacteria are different. In gram positive bacteria, the DNA is taken up as a single stranded molecule and the complementary strand is made in the recipient. In contrast, gram negative bacteria take up double stranded DNA.

Legitimate/homologous/general recombination, after the donor DNA is taken up, a reciprocal recombination event occurs between the chromosome and the donor DNA. This recombination requires homology between the donor DNA and the chromosome and results in the substitution of DNA between the recipient and the donor as illustrated. *E. coli* strains undergoing conjugation. Gene transfers that have been shown to occur between different species of bacteria

Recombination requires the bacterial recombination genes (recA, B and C) and homology between the DNA's involved. This type of recombination is called legitimate or homologous or general recombination. Because of the requirement for homology between the donor and host DNA, only DNA from closely related bacteria would be expected to successfully transform, although in rare instances gene transfer between distantly related bacteria has been shown to occur.

Transduction

Transduction is the transfer of genetic information from a donor to a recipient by way of a bacteriophage. The phage coat protects the DNA in the environment so that transduction, unlike transformation, is not affected by nucleases in the environment. Not all phages can mediate transduction. In most cases gene transfer

is between members of the same bacterial species. However, if a particular phage has a wide host range then transfer between species can occur. The ability of a phage to mediated transduction is related to the life cycle of the phage.

Types of Transduction

Generalized transduction, generalized transduction is transduction in which potentially any bacterial gene from the donor can be transferred to the recipient. The mechanism of generalized transduction is illustrated.Phages that mediate generalized transduction generally breakdown host DNA into smaller pieces and package their DNA into the phage particle by a "head-full" mechanism. Occasionally one of the pieces of host DNA is randomly packaged into a phage coat. Thus, any donor gene can be potentially transferred but only enough DNA as can fit into a phage head can be transferred. If a recipient cell is infected by a phage that contains donor DNA, donor DNA enters the recipient. In the recipient a generalized recombination event can occur which substitutes the donor DNA and recipient DNA.

Specialized transduction, specialized transduction is transduction in which only certain donor genes can be transferred to the recipient. Different phages may transfer different genes but an individual phage can only transfer certain genes. Specialized transduction is mediated by lysogenic or temperate phage and the genes that get transferred will depend on where the prophage has inserted in the chromosome.

During excision of the prophage, occasionally an error occurs where some of the host DNA is excised with the phage DNA. Only host DNA on either side of where the prophage has inserted can be transferred (i.e. specialized transduction). After replication and release of phage and infection of a recipient, lysogenization of recipient can occur resulting in the stable transfer of donor genes. The recipient will now have two copies of the gene(s) that were transferred. Legitimate recombination between the donor and recipient genes is also possible.

Conjugation

Transfer of DNA from a donor to a recipient by direct physical contact between the cells. In bacteria there are two mating types a donor (male) and a recipient

(female) and the direction of transfer of genetic material is one way; DNA is transferred from a donor to a recipient.

Mechanism of conjugation

Pair formation, the tip of the sex pilus comes in contact with the recipient and a conjugation bridge is formed between the two cells. It is through this bridge that the DNA will pass from the donor to the recipient. Thus, the DNA is protected from environmental nucleases. The mating pairs can be separated by shear forces and conjugation can be interrupted. Consequently, the mating pairs remain associated for only a short time.

DNA transfer, the plasmid DNA is nicked at a specific site called the origin of transfer and is replicated by a rolling circle mechanism. A single strand of DNA passes through the conjugation bridge and enters the recipient where the second strand is replicated.

This process explains the characteristics of F+ X F- crosses. The recipient becomes F+, the donor remains F+ and there is low frequency of transfer of donor chromosomal genes. Indeed, as depicted in Figure 7 there is no transfer of donor chromosomal genes. In practice however, there is a low level of transfer of donor chromosomal genes in such crosses.

4. Type of Antimicobials (53).

Antimicrobials are generally used to treat bacterial infections. The toxicity to humans and other animals from antimicrobials is generally considered to be low. However, prolonged use of certain antimicrobials can decrease the number of gut flora, which can have a negative impact on health. Some recommend that during or after prolonged antimicrobials use, that one should consume probiotics and eat reasonably to replace destroyed gut flora.

The term antimicrobials originally described only those formulations derived from living organisms but is now applied also to synthetic antimicrobials, such as the sulfonamides.

The discovery, development, and clinical use of antimicrobials during the 20th century has substantially decreased mortality from bacterial infections. The

antimicrobials era began with the pneumatic application of nitroglycerine drugs, followed by a “golden” period of discovery from approximately 1945 to 1970, when a number of structurally diverse, highly effective agents were discovered and developed. However, since 1980 the introduction of new antimicrobial agents for clinical use has declined. Paralleled to this there has been an alarming increase in bacterial resistance to existing agents.

antimicrobials are among the most commonly used drugs. For example, 30% or more hospitalized patients are treated with one or more courses of antibiotic therapy. However, antibiotics are also among the drugs commonly misused by physicians, e.g. usage of antibiotic agents in viral respiratory tract infection. The inevitable consequence of widespread and injudicious use of antimicrobials has been the emergence of antimicrobials -resistant pathogens, resulting in the emergence of a serious threat to global public health. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antimicrobials. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals.

Although there are several classification schemes for antibiotics, based on bacterial spectrum (broad versus narrow) or route of administration (injectable versus oral versus topical), or type of activity (bactericidal vs. bacteriostatic), the most useful is based on chemical structure. Antimicrobials within a structural class will generally show similar patterns of effectiveness, toxicity, and allergic potential.

4.1 Penicillins.

The penicillins are the oldest class of antibiotics, and have a common chemical structure which they share with the cephalopsorins. Then two groups are classed as the beta-lactam antibiotics, and are generally bacteriocidal that is, they kill bacteria rather than inhibiting growth. The penicillins can be further subdivided. The natural pencillins are based on the original penicillin G structure; penicillinase-resistant penicillins, notably methicillin and oxacillin, are active even in the presence of the bacterial enzyme that inactivates most natural penicillins. Aminopenicillins such as ampicillin and amoxicillin have an extended spectrum of action compared with the natural penicillins; extended spectrum penicillins are effective against a wider range of

bacteria. These generally include coverage for Pseudomonas aeruginaosa and may provide the penicillin in combination with a penicillinase inhibitor.

4.2 Cephalosporins.

Cephalosporins and the closely related cephamycins and carbapenems, like the pencillins, contain a beta-lactam chemical structure. Consequently, there are patterns of cross-resistance and cross-allergenicity among the drugs in these classes. The "cepha" drugs are among the most diverse classes of antibiotics, and are themselves subgrouped into 1st, 2nd, 3rd, 4nd generations. Each generation has a broader spectrum of activity than the one before. In addition, cefoxitin, a cephamycin, is highly active against anaerobic bacteria, which offers utility in treatment of abdominal infections. The 3rd generation drugs, cefotaxime, ceftizoxime, ceftriaxone and others, cross the blood-brain barrier and may be used to treat meningitis and encephalitis. Cephalopsorins are the usually preferred agents for surgical prophylaxis.

4.3 Fluroquinolones.

The fluroquinolones are synthetic antibacterial agents, and not derived from bacteria. They are included here because they can be readily interchanged with traditional antibiotics. An earlier, related class of antibacterial agents, the quinolones, were not well absorbed, and could be used only to treat urinary tract infections. The fluroquinolones, which are based on the older group, are broad-spectrum bacteriocidal drugs that are chemically unrelated to the penicillins or the cephaloprosins. They are well distributed into bone tissue, and so well absorbed. So in generally, the efficacy of oralrouts is the intravenous infusion.

4.4 Tetracyclines.

Tetracyclines got their name because they share a chemical structure that has four rings. They are derived from a species of Streptomyces bacteria. Broad spectrum bacteriostatic agents, the tetracyclines may be effective against a wide variety of microorganisms, including rickettsia and amebic parasites.

4.5 Macrolides.

The macrolide antibiotics are derived from Streptomyces bacteria, and got their name because they all have a macrocyclic lactone chemical structure. Erythromycin, the prototype of this class, has a spectrum and use similar to penicillin. Newer members of the group, azithromycin and clarithyromycin, are particularly useful for their high level of lung penetration. Clarithromycin has been widely used to treat Helicobacter pylori infections, the cause of stomach ulcers.

4.6 Others.

Other classes of antibiotics include the aminoglycosides, which are particularly useful for their effectiveness in treating *Pseudomonas aeruginosa* infections

4.7 Cefoperazone/sulbactam (54)

Cefoperazone/sulbactam is cefoperazone combination with sulbactam. cefoperazone is third generation cephalosporin and sulbactam is a derivative of the basic penicillin nucleus.

Cefoperazone is active in vitro against a wide range of aerobic and anaerobic, gram positive and gram negative pathogens. The bactericidal action of cefoperazone results from the inhibition of bacterial cell wall synthesis. Cefoperazone has a high degree of stability in the presence of beta lactamases produced by most gram negative pathogens. Cefoperazone is usually active against organisms which are resistant to other beta-lactam antibiotics because of beta lactamase production. Cefoperazone is usually active against the following organisms in vitro and in clinical infections.

Sulbactam is a molecule which is given in combination with beta-lactam antibiotics to inhibit beta-lactamase, an enzyme produced by bacteria that destroys the antibiotics. Sulbactam is an irreversible inhibitor of beta-lactamase; it binds the enzyme and does not allow it to interact with the antibiotic. Sulbactam is able to inhibit the most common forms of beta-lactamase but is not able to interact with the ampC cephalosporinase. Thus, it confers little protection against bacteria such as

Pseudomonas aeruginosa, *Citrobacter*, *Enterobacter*, and *Serratia*, which often express this gene.

In summarizing we can say that, cefoperazone exerts its bactericidal effect by inhibiting the bacterial cell wall synthesis, and sulbactam acts as a beta lactamase inhibitor, to increase the antibacterial activity of cefoperazone against beta-lactamase producing organisms.

Result of study about of efficancy for cefoperazone-sulbactam by division of infectious disease, Sao Paulo University Hospital, Brazil. Recent studies have highlighted the emergence of infections involving multidrug-resistant *A. baumannii* clinical isolated. Sulbactam offers direct antimicrobial activity against *A. baumannii* species. Accordingly, co-administration of sulbactam with cefoperazone offers the potential of effective empirical therapy against *Acinetobacter* institutions in which they are susceptible (17). In 1996, China had to evaluate the efficacy and safety of cefoperazone/sulbactam coparited with tienam in the treatment of resperiatory tract infections. The overall clinical efficacy rates of cefoperazone/sulbactam and tienam were 93.5% and 93.3%, respectively. The susceptibility rate of bacteria isolated to two drugs: cefoperazone/sulbactam, tienam, were 95.9%, 93.9% respectively (18). In Korea had study to compare the outcome for patients with *Acinetobacter* bacteriamia treated with cefoperazone/sulbactam versus tienem . The mortality rate was found to be as useful as tienem for treating patients with *Acinetobacter* bacteremia (19).

5. Antimicrobial Susceptibility Testing (55)

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include; diffusion: Stokes method, Kirby-Bauer method; dilution: Minimum Inhibitory Concentration were broth dilution and agar dilution; diffusion & dilution: E-test method**.**

Disk Diffusion

Reagents for the Disk Diffusion Test

1. Müeller-Hinton Agar Medium

Of the many media available, Müeller-Hinton agar is considered to be the best for routine susceptibility testing of nonfastidious bacteria for the following reasons:

It shows acceptable batch-to-batch reproducibility for susceptibility testing.

It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.

It gives satisfactory growth of most nonfastidious pathogens.

A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Although Müeller-Hinton agar is reliable generally for susceptibility testing, results obtained with some batches may, on occasion, vary significantly. If a batch of medium does not support adequate growth of a test organism, zones obtained in a disk diffusion test will usually be larger than expected and may exceed the acceptable quality control limits. Only Müeller-Hinton medium formulations that have been tested according to, and that meet the acceptance limits described in, NCCLS document M62-A7- Protocols for Evaluating Dehydrated Müeller-Hinton Agar should be used.

Disc diffusion methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the CLSI. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here. CLSI is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators etc. It promotes accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost-effective

Interpretative criteria of CLSI are developed based on international collaborative studies and well correlated with MIC’s and the results have corroborated with clinical data. Based on study results CLSI interpretative criteria are revised frequently. CLSI is approved by FDA-USA and recommended by WHO.

Procedure for Performing the Disc Diffusion Test

Inoculum Preparation

Growth Method

The growth method is performed as follows

1. At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.

2. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours)

3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 108 CFU/ml for *E.coli* ATCC 25922. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

Direct Colony Suspension Method

1. As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from a 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used). The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer.

2. This approach is the recommended method for testing the fastidious organisms, *Haemophilus* spp., *N. gonorrhoeae,* and *streptococci*, and for testing *staphylococci* for potential methicillin or oxacillin resistance.

Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.

2. The dried surface of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.

3. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Discs to Inoculated Agar Plates

1. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.

2. The plates are inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied. With the exception of *Haemophilus* spp., streptococci and *N. gonorrhoeae*, the plates should not be incubated in an increased CO2 atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO2 will significantly alter the size of the inhibitory zones of some agents.

Reading Plates and Interpreting Results

1. After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the

nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed. If the test organism is a Staphylococcus or *Enterococcus* spp., 24 hours of incubation are required for vancomycin and oxacillin, but other agents can be read at 16 to 18 hours. Transmitted light (plate held up to light) is used to examine the oxacillin and vancomycin zones for light growth of methicillin or vancomycin-resistant colonies, respectively, within apparent zones of inhibition. Any discernable growth within zone of inhibition is indicative of methicillin or vancomycin resistance.

2. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested. Strains of Proteus spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored. When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter. The sizes of the zones of inhibition are interpreted by referring to Tables 2A through 2I (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement, and the organisms are reported as either susceptible, intermediate, or resistant to the agents that have been tested. Some agents may only be reported as susceptible, since only susceptible breakpoints are given.

6. Impact of drug-resistant *A. baumannii* nosocomial infection

Drug-resistant *A. baumannii* can cause high mortality rate and increase the cost of treatment especially of antibiotics drugs and increase length of hospital stay.

6.1 Mortality

Kwon KT. *et al* (11) studied the impact of imipenem resistance on mortality rate in patients with *A. baumannii* infections and found that 30 day mortality rate was higher in the imipenem-resistant *A. baumannii* group (57.5%) than the imipenem-susceptible *A. baumannii* group (27.5%)($p = 0.007$).

Lee NY. *et al* (12) found that patients with multidrug-resistant bacteremia *A. baumannii* bacteremia had a higher mortality rate and incurred greater medical cost than patients with non- multidrug-resistant bacteremia *A. baumannii*

Jamulitrat S. *et al* (13) identify by using univariate analysis that the mortality rate in patients with imipenem-resistant *A. baumannii* nosocomial infection when compared to patients with imipenem-susceptible *A. baumannii* nosocomial infection were higher 33.8% and 24.1% respectively, yielding an unadjust OR was 1.6 (95%CI= 1.2-2.2)

6.2 Cost

Wilson SJ. *et al* (56) conducted a case-control study to determine the attributable direct costs of multidrug-resistant *A. baumannii* in the burn unit in a public teaching hospital. The mean total hospital cost of patients who acquired multidrug-resistant *A. baumannii* was 98,575 USD higher than that of control patients who had identical burns severity of illness indices ($p < .01$)

Jarvis WR. *et al* (57) found that the estimated average costs of these infections are 558 to 593 USD for each urinary tract infection, 2,734 USD for each surgical site infection, 3,061 to 40,000 USD for each bloodstream infection, and 4,947 dollars for each pneumonia case. Even minimally effective infection control programs are cost-effective. In countries with prospective payment systems based on diagnosis-related groups, hospitals lose from 583 to 4,886 USD for each nosocomial infection.

Lee NY. *et al* (12) studied about clinical and economic impact of multidrug- resistance in nosocomial *A. baumannii* bacteremia and they found that

hospitalization cost is 9,349 and 4,865 USD, respectively. In cases and controls have antibiotic therapy cost 2,257 and 1,610 USD, respectively. Thus bacteremia due to multidrug-resistant *A. baumannii* resulted in 13.4 days of additional hospitalization and 3,758 USD of additional cast, compared with bacteremia due to non multidrug-resistance in nosocomial *A. baumannii* .

6.3 Duration of hospitalization

Jarvis W.R. *et al* (57) found that the excess duration of hospitalization secondary to nosocomial infections has been estimated to be 1 to 4 days for urinary tract infections, 7 to 8.2 days for surgical site infections, 7 to 21 days for bloodstream infections, and 6.8 to 30 days for pneumonia.

In 2005, at tertiary care university teaching hospital Cheng Kung university, Taiwan, to discover between patient with multidrug-resistance *A. baumannii* are cases and patient with non multidrug-resistance *A. baum*annii are controls. After the onset of bacteremia, cases and controls had a significantly different length of hospital stay , means were 54.2 and 34.1 days, respectively.(12)

7. Risk factors related to drug-resistant *A. baumannii* nosocomial infection

7.1Patient factors

Sex

Abbo A. *et al* (58) found that male sex were associate with nosocomial multidrug-resistant *A. baumannini* the OR (Odd Ratio) was 3.84 (95%CI = 1.63-8.99)

Baran G. *et al* (59) identifed female's were at risk for nosocomial imipenem-resistant *A. baumannii* infections ($p < 0.05$).

Bulut C. *et al* (60) identified that male gender that was a risk factors for *A. baumannii* bacteremia ($p < 0.05$)

Wisplinghoff H. *et al* (61) identified females as an independent factor associated with the acquisition of *A. baumannii* bloodstream infection in burn patients ($p = 0.027$), he used multivariate anlysis.

Age

Lee SO. *et al* (62)) found that the age of the patient was associated with nosocomial imipenem-resistant *A. baumannii* infectious, the OR was 1.03 (95%CI=1.01-1.05)**.**

Al Jarousha AM. *et al* (63) found that the age < 7 days of neonates increase risk of multidrug-resistant *A. baumannii* (OR = 2.33, $p = 0.027$).

Co-morbidity

Cardiovascular disease

Abbo A. *et al* (58) found that ischemic heart disease was associated with nosocomial multidrug-resistant *A. baumannii,* an OR was 3.35 (95%CI=1.44-7.77)**.**

Koprnova J. *et al* (64) found that burn patients to were increase the risk of *A. baumannii* bacteremia, OR 5.08 ($p <0.001$).

Baraibar J. *et al* (65) found that patients who had lung aspiration and heart disease associated with infection by *A. baumannii* (p=0.003 and p=0.04), respectively.

Respiratory disease

Garcia Garmendia JL. *et al* (66) found that patients who had chronic obstructive pulmonary diseases compared to non pulmonary disease the RR for *A. baumannii* bacteremia was 2.38 (95%CI= 1.1-4.80).

Immunosuppression disease

GarciaGarmendia JL. *et al* (66) found that patients who had immunosuppression disease compared to non immunosuppression disease the RR for *A. baumannii* bacteremia was 6.90 (95%CI=1.79-26.50)

Neurological disease

Garcia Garmendia JL. *et al* (66) found that patients who had neurological disease compared to non neurological disease the RR for *A. baumannii* bacteremia was 3.26 (95%CI= 1.31-8.13)

Diabetes Mellitus

Alp E. *et al* (67) found that patients were diabetes mellitus increase risk of multidrug-resistant *A. baumannini* (OR=4.14, $p = 0.008$).

7.2 Treatment factors

Duration of admission prior infection

Lee SO. *et al* (62) studied at Asan Medical Center in Seoul , Korea, tertiary – care teaching hospital, to identify the risk factors for nosocomial of acquisition imipenem-resistant *A. baumannii*. The multivariable logistic-regression analysis demonstrated that the time from admission to positive culture date for *A. baumannii* positive patients, was a significant risk factor for the isolation of imipenem-resistant *A.baumannii* (OR = 1.02, 95 %CI = 1.002-1.03) and the risk was greater increase prior stay in the intensive care unite (OR = 21.54 , 95 % CI = 10.73-43.23).

Baran G. *et al* (59) identified the risk factors for nosocomial imipenem-resistant *A.baumannii* infections, by multivariate analysis found that the duration of hospital stay befor *A. baumannii* isolation was the risk (OR = 1.043, 95%CI=1.003-1.084).

Surasarang K. (14) found that patients who had admission > 1 week prior to multidrug-resistant *A.baumannii* nosocomial infection was increase risk (OR= 2.06, 95%CI=1.09-3.89).

Immunosuppressive therapy

Garcia Garmendia JL. *et al* (66) studied the risk factors for *A. baumannii* nosocomial bacteremia and found that patients who received immunosuppressive therapy had the great risk of *A. baumannii* nosocomial infection the OR was 2.99 (95%CI=1.26-7.13).

Koprnova J. *et al* (64) found that antineoplastic chemotherapy had associated with *A. baumannii* bacteremia (p <0.001).

Kwon KT. *et al* (11) studied about the impact of imipenem resistance on the mortality in patients with *A. baumannii* bacteremia and multivariate analysis showed that immunosuppressive status were independent risk factors for 30 day mortality among patients with *A. baumannii* bacteremia (p<0.05).

Chen HP. *et al* (68) found that the factors that independently correlated with the mortality in *A. baumannii* bacteremia were immunosuppressive status (p=0.001).

Mechanical ventilation

Abbo A. *et al* (58) found that patients who had mechanical ventilation were associated with *A. baumannii* nosocomial bacteremia (p < 0.001)**.**

Chen HP. *et al* (68) found that previous mechanical ventilation had associated with mortality in *A. baumannii* bacteremia (p <0.035).

Garcia Gaemedia JL. *et al* (66) found that mechanical ventilation were associated with *A. baumannii* nosocomial bacteremia (p< 0.001)**.**

Simor AE. *et al* (69) found that patients who had mechanical ventilation ≥ 7 days raised an risk for acquisition of multidrug-resistant *A. baumannii* (OR=6.4, p = 0.001)**.**

Bulut C. *et al* (60) identify by multivariate analysis, found that only mechanical ventilation increased the risk for hospital acquired *A. baumannii* bactermia (p < .05).

Jamulitrat S. *et al* (13) identify by univariate analysis and found that mechanical ventilation was the risk factor of imipenem-resistant *A. baumannii* (OR= 1.53, 95%CI=1.13-2.08).

Invasive Procedures

Shih M.J. *et al* (70) found that patients who had recent invasive procedures had the risk for multidrug resistance in nosocomial bactermia due to *A. baumannii* of 4.19 (95%CI=1.6-11.1).

Abbo A. *et al* (58) found that patients who had foley cather increase the risk of *A. baumannii* nosocomial infection OR was 2.42 (95% CI=1.3-4.52, p=0.005).

Simor A.E. *et al* (69) found that patients who had arterial catheter increase the risk for multidrug resistant *A. baumannii* infection in a burn unit, OR 9.2 ($p < 0.001$).

Garcia- Garmendia J.L. *et al* (66) found that patients who had invasive procedures increase the risk of *A. baumannii* nosocomial bacteremia, OR was 1.82 (95% CI=1.38-2.39).

Cisneros J.M. *et al* (71) studied the risk factors for the acquisition of imipenem-resistant *A. baumannii* in Spain, they found that patients who had a urinary catheter increase the risk OR was 2.7 (95% CI=1.1-6.7).

Chen H.S. *et al* (72) found that patients who had central venous catheterization > 7 days, increase the risk for *A. baumannii* nosocomial infection OR 4.493 (95% CI=1.782-11.329).

del Mar Tomas M. *et al* (73), by multivariate analysis, found that the risk factors for infection with *A. baumannii* multidrug resistant was associated with an arterial catheter(OR=1.13,95CI%= 1.03-1.25).

Surasarang K. (14) studied about the risk factors for multidrug resistant *A. baumannii* nosocomial infection and found that the risk increased in patients had indwelling urinary catheter > 1 week (OR=8.2, 95%CI=3.81-17.82), had central venous line > 1 week (OR=3.29, 95%CI=1.48-7.31), nasogastric intubation > 1 week (OR=6.22, 95% CI=3.24-11.93) and central venous line > 1 week raised the risk for multidrug resistant *A. baumannii* nosocomial infections, OR was 3.29 (95% CI= 1.48-7.31).

Wisplinghoff H. *et al* (61) identify by univariate analysis found that central venous line and nasogastric intubation increase risk for nosocomial bloodstream infections due to *Acinetobacter baumannii* (OR= 4.5, 95%CI= 1.7-23.8 and OR= 13.6, 95%CI=1.7-108), respectively.

Katragkou A. *et al* (74) found that by univariate analysis the central venous line was the significant risk factor imipenem-resistant *A. baumannii* nosocomial infection ($p < 0.05$)

Baran G. *et al* (59), identify by univariate, found that the risk factors for nosocomial imipenem-resistant A. baumannii infection were endotracheal tube and nasogastric intubation (p <0.05).

Chaladchalam S. (75) found that bed rail and endotracheal tube connectors were the two most comom site for A. baumannii contamination (36.8%).

Surgery

Lee SO. *et al* (62) identify by bivariate analysis found that surgery treatment increased the risk of imipenem-resistant *A .baumannii* infection (OR= 1.96, 95%CI = 1.26-3.04).

Cisneros JM. *et al* (71) identify by multivarat analysis found that previous surgery was an independent risk factors for acquisition of imipenem- resistant *A. baumannii* (OR=2, 95% CI=1.07-3.8).

Koprnova J. *et al* (64) found the significant relation between to *A. baumannii* bacteremia and surgical (p < 0.05).

Chen HP. *et al* (68) found that *A. baumannii* bacteremia patients who had recent surgery associated with increase mortality (p =0.008).

Baraibar J. *et al* (65) found that the risk for *A. baumannii* nosocomial pneumonia in intubated patients was to be higher in patients with neurosurgery (OR= 10.03, 95%CI = 1.55-64.90)**.**

Villers D. *et al* (76) found that surgical patients were associated with infection when compared with non surgical patients OR 8.5(95% CI = 1.5-63.0) and if undergone surgery in the emergency operating room before the admission to the intensive care unit also increase the risk of infection, OR 5.7(95%CI= 1.07-27.0)**.**

Antimicrobial agents

Garcia Garmendia JL. *et al* (66) studied the risk factors for *A. baumannii* nosocomial bacteremia and found that the patients who had previous antimicrobial therapy increased risk 2.35 times (95% CI=1.10-5.03)

Shih MJ. *et al* (70) studied the risk factors of multidrug-resistance in nosocomial *A. baumannii* bacteremia and found that the number of recently prescribed antibiotics was the risk factor, OR of 1.35 (95% CI=1.0-1.8).

Baran G. *et al* (59) found that patients who had previous antibiotic use increase the risk for imipenem-resistant *A. baumannii* nosocomial infection OR 5.051 (95%CI=1.004-25.396, p =0.049).

Koprnova J. *et al* (64) found that prior antibiotic therapy associated with *A. baumannii* bacteremia (p<0.001).

Nseir S. *et al* (77) found that the patients who had prior antibiotic treatment in the ICU increase risk acquired multidrug-resistant bacteremia (OR=1.9, 95%CI=1.0-3.6).

Surasarang K. (14) conducted a case-control study at Siriraj Hospital to indentify the risk factor for the nosocomail associated of multidrug-resistant *A. baumannii* and found the antibiotic use. The following were associated with increase risk of infection; 3rd - 4th generation cephalosprins (OR=1.80, 95 %CI= 1.21-5.56) and piperacillin plus tazobactam (OR=4.68, 95%CI=1.93-11.32).

Corbella X. *et al* (78) found that those patients who had previously received therapy with carbapenems the relative risk (RR) for carbapenem resistance *A. baumannii* was 4.6 (95% CI= 1.3-15.6).

Lee SO. *et al* (62) found that the patients who prior exposure to imipenem had higher risk for acquisition imipenem-resistant *A. baumannii* (OR= 9.18, (95% CI= 3.99-21.13) or third generation cephalosporins (OR= 2.11, 95%CI=1.13-3.95) and by univariate analysis presented to vancomycin use associated with acquisition of imipenem-resistant *A. baumannii* (OR=9.27, 95%CI=4.99-17.28).

Cisneros JM. *et al* (71) studied risk factors for the acquisition of imipenem resistant *A. baumannii* in Spain, found that patients who had previous antimicrobial treatment the OR was 4.3 (95%CI1.6-11).

Manikal VM. *et al* (15) found that antibiotic usage data from 11 hospitals revealed that the use of third generation cephalosporins was associated significantly with the percentage of carbapenem resistant strains (p =0.03).

Abbo A. *et al* (58) found that protective effect from multidrug-resistant *A. baumannii* infection in patients who had penicillin use (OR= 0.38, 95% CI= 0.16-0.90, p=0.029).

7.3 Environment factor

Ward

Corbella X. *et al* (78) found that those who were admitted into a ward with a high density of patients increase the risk of cabapenem resistance *A. baumannii* infection (RR=1.7, 95%CI=1.2-2.5).

Lee SO. *et al* (62) found that the risk factors for imipenem-resistant was strongly related with a previous intensive care unit (ICU) stay (OR=21.54, 95% CI=10.73-43.23).

Cisneros JM. *et al* (71) found that independent risk factor for the acquisition of imipenem-resistant *A. baumannii* infection was the size of hospital which langer than 500 bed, by multivariate the OR was 6.5(95%CI=1.8-23).

Baran G. *et al* (59) found that the patients who had ICU stay befor had increase risk of imipenem-resistant *A. baumannii* infection, the OR was 3.1(95%CI=1.398-6.873).

7.4 Co-infection bacteria

Goldstein FW. *et al* (79) studied transferable plasmid-mediated antibiotic resistance in *Acinetobacter*, the results presented also supports the hypothesis that plasmid pIP1031 may have been acquired recently by strain BM2500, *A. calcoaceticus* strain BM2500 was resistant to ampicillin, aminoglycoside-aminocyclitols, chloramphenicol, sulfonamides, and high levels of trimethoprim. Resistance to ampicillin was due to the presence of a beta-lactamase and the aminoglycoside-aminocyclitol resistance was mediated by phosphotransferase and adenylyltransferase activities. The resistance genes were carried by a 167 kilobase plasmid, pIP1031, belonging to incompatibility group 6-C; the plasmid was self-transferable, at extremely low frequency, to *Escherichia coli* by conjugation. Plasmid pIP1031 DNA was analyzed by agarose gel electrophoresis following restriction endonuclease digestion, by nucleic acid hybridization, and by CsCl analytical density gradient ultracentrifugation

Joly Guillou ML. *et al* (80) found that co-infection of different strains or different pathogens caused increase the problem of treatment, especially multidrug-resistan strains.

Shih MJ. *et al* (70) identified by multivariate logistic regression analysis patients who previous have had colonizated with *A. baumannii* were associated with multidrug-resistant *A. baumannii* bacteremia (OR=7.99, 95%CI=2.1-30.6).

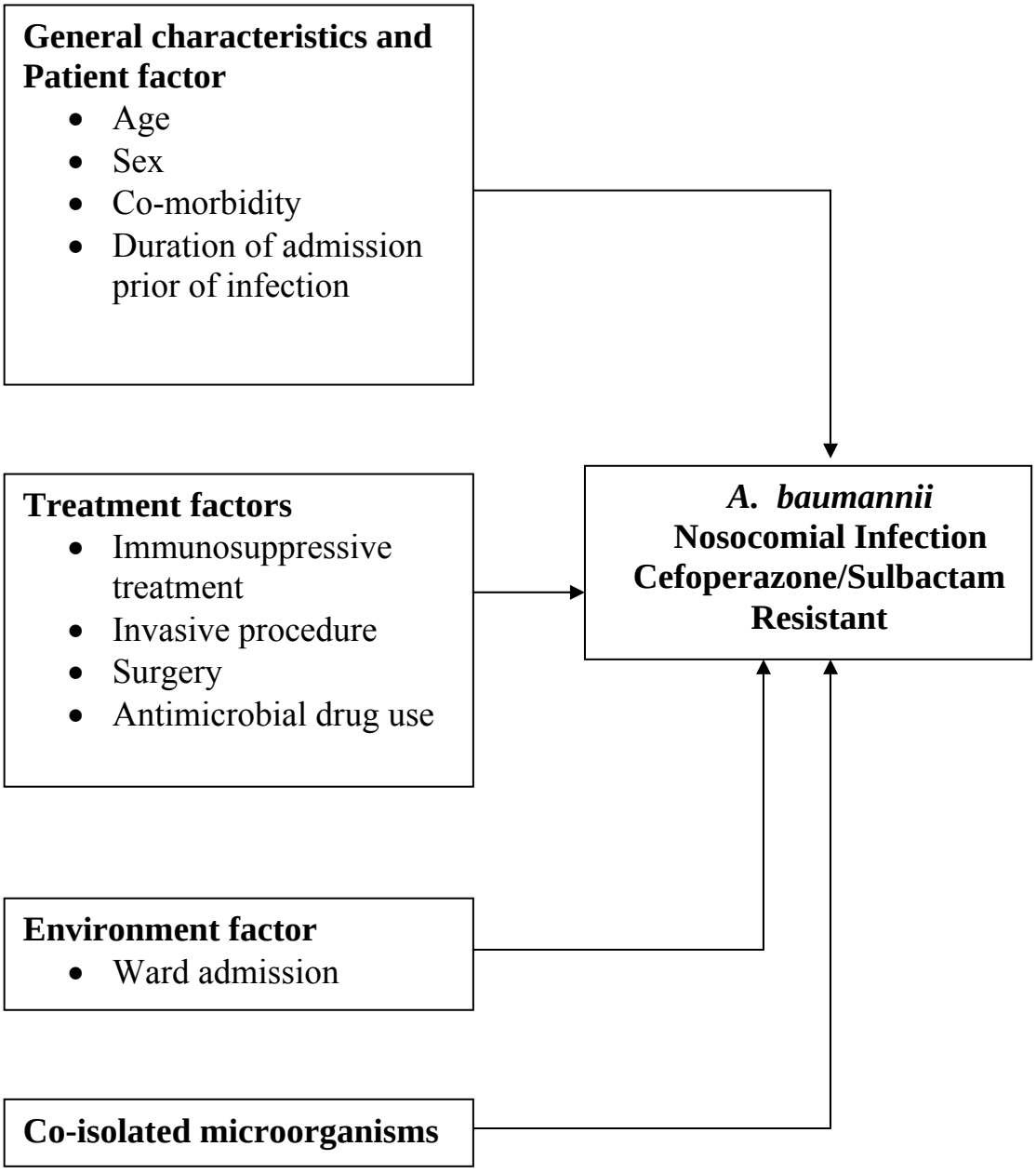


Figure 1 Conceptual framework

CHAPTER III
MATERIALS AND METHODS

1. Study design

Hospital based case control study was conducted to determine the risk factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection.

2. Study Population

Patients who were admitted at Rajavithi Hospital and have *A. baumannii* infection including had taken and cefoperazone/sulbactam sensitivity test from laboratory' Rajavithi Hospital during January 1 to November 30, 2007.

3. Definition of Case Control

Case

Patient who had report from Rajavithi Hospital'laboratory of cefoperazone/sulbactam-resistant *A. baumannii* isolates and identified as nosocomial infection by definition from CDC during January 1 to November 30, 2007.

Control

Patient who had report from Rajavithi Hospital'laboratory of cefoperazone/sulbactam-susceptible *A. baumannii* isolates and were identified as nosocomial infection by definition from CDC during January 1 to November 30, 2007.

Exclusion Criteria

Patient who had incomplete data were excluded from the study.

4. The Sample Size

The sample size was calculated by using case control ratio 1:2

$$n = \frac{[Z\,\alpha/2\sqrt{(c+1)P(1-P)} + Z\,ß\sqrt{cP1(1-P1) + P0(1-P0)}]2}{c(P1-P0)2}$$

$$P1 = \frac{OR \times P0}{OR \times P0 + (1-P0)}$$

$$P = \frac{P1 - c\,P0}{(c+1)}$$

n = number of case to be sampled

Z α = Z value from table standard normal distribution at the specific level of α

Z ß = Z value from table standard normal distribution at the specific level of ß

OR = Odd ratio

P1 = The proportion of sample in cases who exposed

P0 = The proportion of sample in controls who exposed

c = The ratio of control per case

α = 0 .05 ; Zα/2 = 1.96

ß = 0.1 ; Zß = 1.28

c = 2

Table 1 Sample size from calculation

Risk factor	OR	P0	Number of case	Number of control	Total	Reference
Co-morbidity	3.35	0.5	49	98	147	(58)
Duration of admission prior of infection	2.65	0.5	70	140	210	(13)
Antimicrobial treat	2.11	0.3	15	30	45	(62)
Mechanical ventilation	6.27	0.4	29	58	87	(58)
Invasive procedure	2.49	0.6	142	284	426	(58)
Immunosuppressive treatment	1.1	0.3	15	30	45	(58)
Surgery	1.96	0.3	15	30	45	(62)
ICU stay	21.5	0.1	4	8	12	(13)

Therefore, with the minimum OR of 2.49 and the risk of invasive procedures, the maximum sample size was 142 for cases and 284 for controls. The total minimum of sample size 427 patients was calculated for the study.

5. Research Tools

The research instrument was the data collection form prepared by the investigator. It consisted of 3 parts:

Part 1: General characteristics

Age

Sex

Occupation

Principle diagnosis

Admission date

Discharge date

Discharge status

Patent

Part 2 *A. baumannii* nosocomial infection

Site of infection

Type Specimens

Part 3 Risk factors

Date sent specimens

Ward admitted

History of ward /hospital transfer

Co-morbidity

Immunosuppressive treatment

Invasive procedure

Surgery

Antimicrobial drug taken

Co-isolated microorganisms

6. Methods of Data Collection

Data was collected from the patients records and included laboratory reports of Rajavithi Hospital.

Cases were collected from those having laboratory reports of cefoperazone/sulbactam-resistant *A. baumannii* isolates and further identified as nosocomial infection by definition from CDC during January 1 to November 30, 2007.

Controls were selected in the same way as the cases, but only among those were infected with cefoperazone/sulbactam-susceptible *A. baumannii* isolates, from January 1 to November 30, 2007.

General characteristics of cases and controls was collected at initial stage and later medical information collected from patient records, namely the inpatient department (IPD) card.

7. Statistical Analysis

Descriptive statistics including frequencies, percentage, mean and standard deviation were used to describe the patient characteristics of the study population.

Analytic statistics:

Chi-square test and t-test were used to find the association between the general characteristics (age, gender, duration of admission in hospital, case fatality rate) between case and control group.

Univariate analysis was used to assess the crude odd ratio (crude OR) between patient factors, treatment factors, environment factors and co-isolated microorganisms factor association with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection.

Multivariate analysis was used to assess the adjusted odd ratio (adjusted OR) between patient factors, treatment factors, environment factors and co-isolated microorganisms factor association cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection.

8. Ethical consideration

This study was approved by the Committee on Research Involving Human Subjects Rajavithi Hospital, Bangkok.

CHAPTER IV
RESULT

The study was carried out at Rajavithi Hospital to determine the risk factors of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection among patients admitted in Rajavithi Hospital during January 1 to November 30, 2007. The study population was selected from 679 patients with *A. baumannii* isolated from their clinical specimen. There were 148 cases fo cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection and 295 controls of cefoperazone/sulbactam-susceptible *A. baumannii* nosocomial infection in the analysis.

1. Incidence of cefoperazone/sulbactam-resistant *A. baumannii* infection among the patients admitted at Rajavithi Hospital.

There was 679 cases of *A. baumannii* isolated from first submitted clinical specimens from January 1 to November 30, 2007 at Rajavithi Hospital. Table 2 shows distribution of cefoperazone/sulbactam-resistant *A. baumannii* (218 cases) and cefoperazone/sulbactam-susceptible *A. baumannii* (461 cases) by month during the study period.

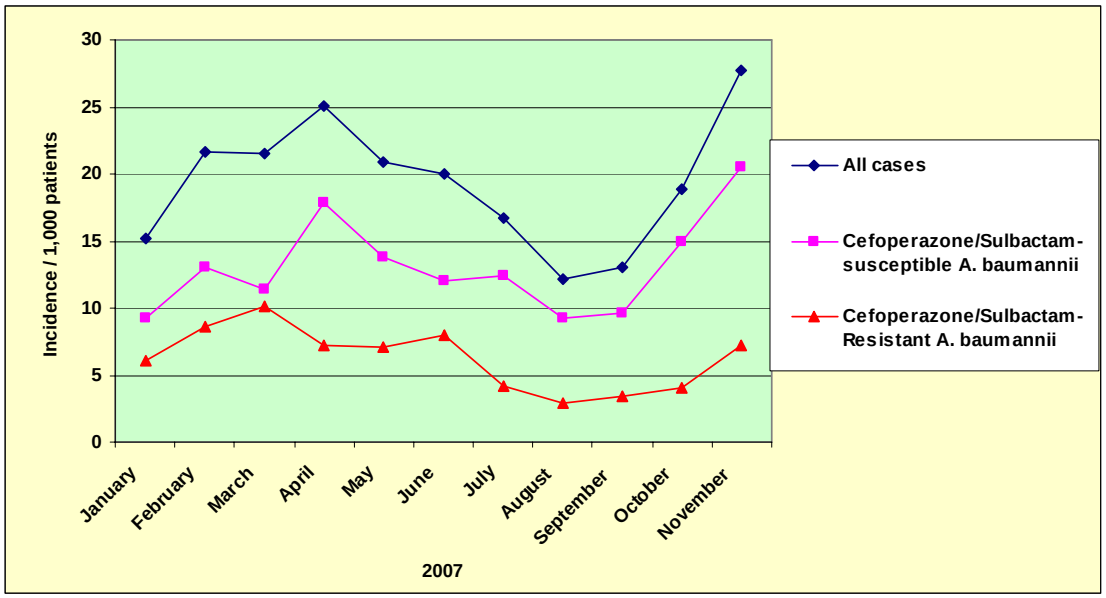
The incidence of *A. baumannii* infection during the study period was 60.2/1,000 patients. The incidences were increase from 15.2/1,000 patients in January to 25.1/1,000 patients in April. Then, the incidence gradually declined to 12.2/1,000 patients in August. The incidence of cefoperazone/sulbactam-susceptible *A. baumannii* infection during the study period was 13.0/1,000 patients. The incidences by month were similar to incidence of *A. baumannii* infection. (Figure 1)

The incidence of cefoperazone/sulbactam-resistant *A. baumannii* infection (during the study period were 6.2 per 1,000 patients).The highest incidence was found in March (10.1/1,000 patients) and lower in August (2.9/1,000 patients)(Table 2).

Table 2 Incidence (per 1,000 patients) of *A. baumannii* infection in 2007 by month.

Month	Patients admission	Total		cefoperazone/sulbactam-susceptible		cefoperazone/sulbactam-resistant	
		Number	Incidence	Number	Incidence	Number	Incidence
January	3,281	50	15.2	30	9.1	20	6.1
February	2,903	63	21.7	38	13.1	25	8.6
March	3,169	68	21.5	36	11.4	32	10.1
April	2,906	73	25.1	52	17.9	21	7.2
May	3,117	65	20.9	43	13.8	22	7.1
June	3,247	65	20	39	12	26	8
July	3,299	55	16.7	41	12.4	14	4.2
August	3,447	42	12.2	32	9.3	10	2.9
September	3,221	42	13	31	9.6	11	3.4
October	3,542	67	18.9	53	15.0	14	4
November	3,218	89	27.7	66	20.5	23	7.2
Total	35,350	679	19.2	461	13.0	218	6.2

Figure 2 Incidences (per 1,000 patients) of *A. baumannii* infection in 2007 by month



2. General characteristics of the patients infected with sulperazone-resistant *A. baumannii*.

Among 679 cases, only 443 nosocomial infections with completed data were included in the analysis. According to case and control definitions, there were 148 cases of cefoperazone/sulbactam-resistant *A. baumannii* and 295 controls of cefoperazone/sulbactam-susceptible *A. baumannii* nosocomial infection. Table 3 shows general characteristics of the study population. More than 60 % of cases and 51% of controls were older than 60 years old. About 58.8 % of cases and 55.3% of controls were male. The patients' age in the cases group was ranged from 1 to 96 years while the controls group the age ranged from 1 to 103 years with means 62.56 (SD = 19.6 years) and 58.31 (SD = 20.3 years), respectively.

The fatality rate of case group (62.2%) was significant difference than from between those of control group 42.0% ($p < 0.001$). The mean length of hospital stay in case group 7.80 weeks (SD=7.28 weeks) was higher than control group 6.89 weeks (SD=7.35 weeks). About 53.4% of case group and control group 50.8% were houseworker. (Table 3)

Table 3 General characteristics of cefoperazone/sulbactam-resistant *A. baumannii* and cefoperazone/sulbactam-susceptible *A. baumannii* nosocomial infection.

Characteristics	Total	Case (148)		Control (295)		*p*-value
		Number	%	Number	%	
Sex						0.480
Male	250	87	58.8	163	55.3	
Female	193	61	41.2	132	44.7	
Age (years)						0.294
0-15	10	3	2.0	7	2.4	
16-30	35	10	6.8	25	8.5	
31-45	54	12	8.1	42	14.2	
46-60	104	34	23.0	70	23.7	
>60	240	89	60.1	151	51.2	
Range of age		1-96 years		1-103 years		
Mean (± SD)		62.56 (±19.6)		58.31 (±20.3)		
Death	216	92	62.2	124	42.0	<0.001*
0-15	1	0	0	1	0.3	
16-30	16	7	4.7	9	3.1	
31-45	17	6	4.1	11	3.7	
46-60	50	20	13.5	30	10.2	
>60	132	59	39.9	73	24.7	
Fatality rate		62.2		42.0		
Length of hospital stays						0.119
1-2 weeks	96	25	16.9	71	24.1	
3-4 weeks	113	33	22.3	80	27.1	
5-6 weeks	69	28	18.9	41	13.9	
7-8 weeks	47	19	12.8	28	9.5	
9-10 weeks	33	13	8.8	20	6.8	
≥ 11 weeks	85	30	20.3	55	18.6	
Rang		1-43 weeks		1-47 weeks		
Mean (±SD)		7.80 (±7.28)		6.89 (±7.35)		
Occupations						
Housework	229	79	53.4	150	50.8	
State enterprise	123	39	26.4	84	28.5	
Government	35	11	7.7	24	8.1	
Business	27	11	7.4	16	5.4	
Student	16	5	3.4	11	3.7	
Agriculture	12	3	2.0	10	3.4	

*statistical significane at $\alpha < 0.05$

3. Type of specimens and microorganisms co-isolated with *A. baumannii*.

Regarding types of specimens that *A. baumannii* were isolated, sputum and tracheal secretion were the most common specimens and accounted for 62.8% and 57.6 % of cases and control specimens , respectively. Wound pus was the second most common specimen (11.5%), followed by urine (10.1%) among the cases specimens. However, urine was the second most common specimen that control were isolated (15.3%), followed by wound pus (8.1%). (Table 4)

The five most common microorganisms isolated with *A. baumannii* in case group were *Escherichia coli* (33.1%), *Pseudomonas aeruginosa* (27.7%), *Klebsiella neumoniae* (18.9%), *S. aureus* (13.5%) and *E. faecium* (8.8%). (Table 4)

Table 4 Type of specimens and microorganisms isolated with *A. baumannii*.

Variable	Total	Case Number (%)	Control Number (%)
Type of specimens			
Sputum/Tracheal secretion	263	93(62.8)	170(57.6)
Urine	60	15(10.1)	45(15.3)
Wound pus	41	17(11.5)	24(8.1)
Blood	32	13(8.8)	19(6.4)
Pleural fluid	6	2(1.4)	4(1.4)
Peritoneal	3	1(0.7)	2(0.7)
Cerebrospinal fluid	2	1(0.7)	1(0.3)
Others	36	6(4.1)	30(10.2)
Total	443	148(100)	295(100)
Co-isolated microorganisms			
E. coli	102	49(33.1)	53(18.0)
P. aeruginosa	89	41(27.7)	48(16.3)
K. pneumoniae	84	28(18.9)	56(19.0)
S. aureus	46	20(13.5)	26(8.8)
E. faecium	40	13(8.8)	27(9.2)
Coag Neg staphylococci*	37	14(9.5)	23(7.8)
Enterobacter spp.	24	3(2.0)	21(7.1)
S. maltophilia	23	11(7.4)	12(4.1)
E. faecalis	20	12(8.1)	8(2.7)
S. hemolyticus	18	8(5.4)	10(3.4)
Proteus spp.	12	6(4.1)	6(2.0)
Others microorganisms	52	22(14.9)	30(10.2)
Total	547	227(100)	320(100)

* Coagulase negative staphylococci

4. Factors associated with Cefoperazone/sulbactam-resistant *A.baumannii* nosocomial infection

The result from univariate analysis of variance among potential risk factors and cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection were as the following:

Patient factors

Duration of admission prior to infection

Risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection among patients who had duration of admission long than 1 week was 1.70 (95% CI = 1.14-2.54) times higher than patients who had duration of admission prior to infection $\leq$ 1 week. (Table 5)

Co-morbidity

The patients who had co-morbidity were found to have higher risk of having cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 2.00, 95% CI = 1.21-3.13). Malignancy (OR=2.78, 95% CI = 1.50-5.134) and chronic renal failure (OR=2.53, 95%CI= 1.36-4.71) were significantly associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. Diabetes mellitus, neurological disease, anemia, liver disease, lung disease, hypertension and old CVA were found to have higher risk of having cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 1.204, 2.468, 1.147, 1.147, 1.828, 1.350 and 1.348), respectively, but no statistical significance. HIV and heart disease were not associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. (Table 5)

Table 5 Patient factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection.

Risk factor	Case		Control		Crude OR	95% CI	*p-value*
	n	%	n	%			
Sex							
Male	87	58.8	163	55.3	1.55	0.77-1.72	0.545
Female	61	41.2	132	44.7	1		
Age							
≤ 60	59	39.9	144	48.8	1		
> 60	89	60.1	151	51.2	1.43	0.96-2.15	0.093
Duration of admission prior to infection							
≤ 1 weeks	73	49.3	184	62.4	1		
> 1 weeks	75	50.7	111	37.6	1.70	1.14-2.54	0.012*
Co-morbidity							
No	29	19.6	95	32.2	1		
Yes	119	80.4	200	67.8	2.00	1.21-3.13	0.007*
Diabetes mellitus							
No	107	72.3	223	75.9	1		
Yes	41	27.7	71	24.1	1.20	0.77-1.88	0.487
HIV							
No	146	98.6	287	97.3	1		
Yes	2	1.4	8	2.7	0.49	0.10-2.34	0.569
Malignancy							
No	122	82.4	274	92.9	1		
Yes	26	17.6	21	7.1	2.78	1.50-5.14	0.001*
Neurological diseases							
No	141	95.9	290	98.3	1		
Yes	6	4.1	5	1.7	2.47	0.74-8.23	0.233
Anemia							
No	136	91.9	273	92.9	1		
Yes	12	8.1	21	7.1	1.15	0.55-2.40	0.863
Chronic renal failure							
No	124	83.8	274	92.9	1		
Yes	24	16.2	21	7.1	2.53	1.36-4.71	0.005*
Heart disease							
No	134	90.5	254	86.1	1		
Yes	14	9.5	41	13.9	0.67	0.34-1.23	0.237
Liver disease							
No	140	94.6	281	95.3	1		
Yes	8	5.4	14	4.7	1.15	0.47-2.80	0.945

OR = Odd Ratio

*statistical significane at $\alpha < 0.05$

Table 5 Patient factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. (cont.)

Risk factor	Case		Control		Crude OR	95% CI	*p-value*
	n	%	n	%			
Lung disease							
No	129	87.2	273	92.5	1		
Yes	19	12.8	22	7.5	1.83	0.96-3.50	0.095
Lung disease							
No	129	87.2	273	92.5	1		
Yes	19	12.8	22	7.5	1.83	0.96-3.50	0.095
Hypertension							
No	88	59.5	195	66.1	1		
Yes	60	40.5	100	33.9	1.35	0.89-2.00	0.205
Old CVA							
No	140	94.6	283	95.9	1		
Yes	8	5.4	12	4.1	1.35	0.54-3.37	0.691
Other co-morbidity							
No	129	87.2	264	89.8	1		
Yes	19	12.8	30	10.2	1.30	0.70-2.40	0.502

OR = Odd Ratio
*statistical significane at $\alpha < 0.05$

Treatment factors

Immunosuppressive Treatment

The patients who received immunosuppressive treatment were found to have higher risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 1.21) but no statistic significance. The patients who received dexamethasone treatment had higher risk (OR=1.615, 95%CI= 1.039-2.510) of cefoperazone/sulbactam-resistant than those who did not receive dexamethasone (Table 6).

Invasive procedure

The patients who received invasive procedure had higher risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 2.45, 95% CI= 1.06-5.69) when compared with those without invasive procedure.

The patients who retained urinary catheter when compared with those without urinary catheter had higher risk of cefoperazone/sulbactam-resistant . The patients who retained urinary catheter ≤ 1 week had high risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection more than patients without urinary group (OR = 1.45) but no statistical significance. The patients retianed urinary catheter > 1 week had higher risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 2.75, 95% CI= 1.72-4.40) when compared with patients without urinary catheter (Table 6).

The patients on mechanical ventilation ≤ 1 week when compared with patients without mechanical ventilation, found to have higher risk of having cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 1.91, 95%CI = 1.18-3.10), moreover, patients who on mechanical ventilation for longer time such as > 1 week had increased risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 2.51, 95% CI= 1.54-4.07) compared with who those without mechanical ventilation (Table 6).

The patients on central venous line ≤ 1 week had higher risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 1.30), but no statistical significance when compared with those without central venous line. Moreover, patients who on central venous line for long time (>1 week) had increased risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 3.06, 95%CI = 1.85-5.06) when compared with those without central venous line (Table 6).

The patients with nasogastric intubation ≤ 1 week had increased the risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 2.13, 95% CI = 1.25-3.62) and also in group who on nasogastric intubation > 1 week (OR = 3.51, 95% CI = 2.20-5.98) compared with those without nasogastric intubation (Table 4).

Surgery

The patients who had went to surgical were not associated with cefoperazone/ sulbactam-resistant *A. baumannii* nosocomial infection (OR=0.94, 95%CI= 0.62-1.42) (Table6).

Table 6 Treatment factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection.

Risk factor	Case		Control		Crude OR	95% CI	*p-value*
	n	%	n	%			
Immunosuppressive treatment							
No	93	62.8	198	67.1	1		
Yes	55	37.2	97	32.9	1.21	0.80-1.82	0.430
Prednisolone							
No	137	92.6	274	92.9	1		
Yes	11	7.4	21	7.1	1.05	0.49-2.24	0.904
Dexamethasone							
No	101	68.2	229	77.6	1		
Yes	47	31.8	66	22.4	1.62	1.04-2.51	0.043*
Chemotherapy							
No	143	96.6	284	96.3	1		
Yes	5	3.4	11	3.7	0.90	0.31-2.65	1.00
Invasive procedures							
No	7	4.7	32	10.8	1		
Yes	141	95.3	263	89.2	2.45	1.06-5.69	0.049*
Retained Urinary catheter							
No	43	29.1	137	46.4	1		
≤ 1 weeks	35	23.6	77	26.1	1.45	0.86-2.45	0.168
> 1 weeks	70	47.3	81	27.5	2.75	1.72-4.40	<0.001*
Mechanical ventilation							
No	53	35.8	162	54.9	1		
≤ 1 weeks	45	30.4	72	24.4	1.91	1.18-3.10	0.009*
> 1 weeks	50	33.8	61	20.7	2.51	1.54-4.07	<0.001*
Central venous line							
No	28	18.9	102	34.6	1		
≤ 1 weeks	31	20.9	87	29.5	1.30	0.72-2.33	0.383
> 1 weeks	89	60.1	106	35.9	3.06	1.85-5.06	<0.001*
Nasogastric intubation							
No	48	32.4	171	58.0	1		
≤ 1 weeks	34	23.0	57	19.3	2.13	1.25-3.62	0.005*
> 1 weeks	66	44.6	67	22.7	3.51	2.20-5.98	<0.001*
Surgery							
No	95	64.2	185	62.7	1		
Yes	53	35.8	110	37.3	0.94	0.62-1.42	0.842

OR = Odd Ratio

*statistical significane at $\alpha < 0.05$

Antimicrobial agents

The patients who took carbapenem > 1 week, increased the risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 2.24, 95%CI = 1.27-3.95)when compared with patients did not take carbapenem(Table 7).

The patients who received vancomycin ≤ 1 week had increased risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 2.84,95%CI = 1.290-6.259). The patients who took vancomycin > 1 week also had increased risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR=2.14, 95%CI=1.05-4.38) when compared with those who did not take vancomycin (Table 7).

The patients who used antimicrobial agents≤ 1 week: normaly cabapenem, quinolone, aminoglycoside, first generation cephalosporin, third generation cephalosporin, forth generation cephalosporin, sulfomamide, metronidazole, sulperazone and fosfomycin were found to have higher risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 1.62, 1.41, 1.74, 1.18, 1.13, 2.43, 2.56, 1.60, 1.56 and 1.58), respectively, but no statistical significance. The patients who received antimicrobial > 1 week; quinolone, aminoglycoside, cephalosporin 1st ,macrolide, sulfonamide, metronidazole and cefoperazone/sulbactam were found to have higher risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections (OR = 1.274, 1.477 , 2.029, 1.812, 1.282, 1.870 and 1.633) respectively, but no statistical significance (Table 7).

Table 7 Use of antimicrobial agents associated with cefoperazone/sulbactam resistant *A. baumannii* nosocomial infection.

Risk factor	Case		Control		Crude OR	95% CI	*p-value*
	n	%	n	%			
Antimicrobial drugs use							
No	6	4.1	13	4.4	1		
Yes	142	95.9	282	95.6	1.09	0.41-2.93	1.00
Carbapenem							
No	95	64.2	228	77.3	1		
≤ 1 week	25	16.9	37	12.5	1.62	0.93-2.84	0.091
> 1 week	28	18.9	30	10.2	2.24	1.27-3.95	0.005*
Penicillin							
No	119	80.4	231	78.3	1		
≤ 1 week	21	14.2	47	15.9	0.87	0.50-1.52	0.168
> 1 week	8	5.4	17	5.8	0.91	0.38-2.18	0.838
Quinolone							
No	98	66.2	214	72.5	1		
≤ 1 week	29	19.6	45	15.3	1.41	0.83-2.38	0.202
> 1 week	21	14.2	36	12.2	1.27	0.71-2.30	0.421
Aminoglycosides							
No	127	85.8	268	90.8	1		
≤ 1 week	14	9.5	17	5.8	1.74	0.83-3.64	0.142
> 1 week	7	4.7	10	3.8	1.48	0.55-3.97	0.439
First generation Cephalosporin							
No	138	93.2	280	94.9	1		
≤ 1 week	7	4.7	12	4.1	1.18	0.46-3.07	0.729
> 1 week	3	2.0	3	1.0	2.03	0.40-10.18	0.390
Third generation Cephalosporin							
No	60	40.5	124	42.0	1		
≤ 1 week	55	37.2	101	34.2	1.13	0.71-1.77	0.607
> 1 week	33	22.3	70	23.7	0.97	0.58-1.63	0.921
Fourth generation Cephalosporin							
No	141	95.3	286	96.9	1		
≤ 1 week	6	4.1	5	1.7	2.43	0.73-8.11	0.148
> 1 week	1	0.7	4	1.4	0.51	0.06-4.58	0.545
Macrolide							
No	131	88.5	267	90.5	1		
≤ 1 week	9	6.1	19	6.4	0.97	0.43-2.19	0.933
> 1 week	8	5.4	9	3.1	1.81	0.68-4.80	0.232

OR = Odd Ratio

*statistical significane at $\alpha < 0.05$

Table 7 Use of antimicrobial agents associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. (cont.)

Risk factor	Case		Control		Crude OR	95% CI	*p-value*
	n	%	n	%			
Sulfonamide							
No	138	93.2	283	95.9	1		
≤ 1 week	5	3.4	4	1.4	2.56	0.68-9.70	0.166
> 1 week	5	3.4	8	2.7	1.28	0.41-3.99	0.668
Metronidazole							
No	115	77.7	253	85.8	1		
≤ 1 week	16	10.8	22	7.5	1.60	0.81-3.16	0.176
> 1 week	17	11.5	20	6.8	1.87	0.94-3.70	0.073
Sulperazone							
No	94	63.5	217	73.6	1		
≤ 1 week	25	16.9	37	12.5	1.56	0.89-2.74	0.121
> 1 week	29	19.6	41	13.9	1.63	0.96-2.78	0.072
Fosfomycin							
No	142	95.9	280	94.9	1		
≤ 1 week	4	2.7	5	1.7	1.58	0.42-5.97	0.502
> 1 week	2	1.4	10	3.4	0.39	0.09-1.82	0.234
Vancomycin							
No	117	79.1	266	90.2	1		
≤ 1 week	15	10.1	12	4.1	2.84	1.29-6.26	0.010*
> 1 week	16	10.8	17	5.8	2.14	1.05-4.38	0.037*

OR = Odd Ratio
*statistical significane at $\alpha < 0.05$

Environment factors

Ward admission

The patients who were admitted in ICU wards had increased risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR= 2.02, 95%CI= 1.22-3.35) (Table 8).

Ward transfer

The patients who had transfered from other wards were found to have higher risk of cefoperazone/sulbactam-resistant *Acinetobacter baumannii* nosocomial infection (OR = 1.439, 95% CI= 0.94-2.13), but no statistical significance (Table 8).

Microorganisms isolated

Risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection among the patients who had other microorganisms isolated together with *A .baumannii* was 2.23 times (95% CI = 1.46 – 3.42) higher than those who had only *A .baumanni* isolated from their clinical specimens (Table 8).

Co-isolation of *Pseudomonas aeruginosa, Escherichia coli*, *Enterococcus faecalis* with *A. baumannii* were associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection significantly OR = 1.97, 95% CI = 1.23-3.17; OR=2.26, 95% CI = 1.44-3.56; OR=3.17, 95% CI = 1.27-7.92, respectively. (Table 7)

The patients who had co-isolated microorganisms with *A. baumannii*: *Proteus* spp., *Staphylococcus aureus, Coagulase negative staphylococci, Staphylococcus hemolyticus* and *Stenotrophomonas maltophilia* were found to have higher of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection(OR = 2.035, 1.617, 1.236, 1.629 and 1.894), respectively, but no statistical significance. *Enterobacter* spp., *Klebsiella* spp. and *Enterococcus faecium* when isolated with *A. baumannii* or prior infection were not associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (Table 8).

Table 8 Environment factors and microorganisms isolated associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection.

Risk factor	Case		Control		Crude OR	95% CI	*p-value*
	n	%	n	%			
Ward admission							
General	82	55.4	192	65.1	1		
Surgery	28	18.9	59	20.0	1.11	0.66-1.87	0.690
ICU	38	25.7	44	14.9	2.02	1.22-3.35	0.006*
Transfer Ward							
No	88	59.5	199	67.5	1		
Yes	60	40.5	96	32.5	1.41	0.94-2.13	0.097
Co-infection microorganisms							
No	41	27.9	136	46.1	1		
Yes	106	72.1	159	53.9	2.23	1.46-3.42	<0.001*
P. aeruginosa							
No	107	72.3	247	83.7	1		
Yes	41	27.7	48	16.3	1.97	1.23-3.17	0.007*
E. coli							
No	99	66.9	242	82.0	1		
Yes	49	33.1	53	18.0	2.26	1.44-3.56	0.001*
Proteus spp.							
No	142	95.9	289	98.0	1		
Yes	6	4.1	6	2.0	2.04	0.65-6.42	0.355
Enterobacter spp.							
No	145	98.0	274	92.9	1		
Yes	3	2.0	21	7.1	0.27	0.08-0.92	0.044
S. aureus							
No	128	86.5	269	91.2	1		
Yes	20	13.5	26	8.8	1.62	0.87-3.00	0.172
K. pneumoniae							
No	120	81.1	239	81.0	1		
Yes	28	18.9	56	19.0	1.00	0.60-2.48	0.678
Coag Neg staphylococci							
No	134	90.5	272	92.2	1		
Yes	14	9.5	23	7.8	1.24	0.67-2.48	0.678
S. hemolyticus							
No	140	94.6	285	96.6	1		
Yes	8	5.4	10	3.4	1.63	0.63-4.22	0.448
E. faecalis							
No	136	91.9	287	97.3	1		
Yes	12	8.1	8	2.7	3.17	1.27-7.92	0.019*

OR = Odd Ratio

*statistical significane at $\alpha < 0.05$

Table 8 Environment factors and microorganisms isolated associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. (cont.)

Risk factor	Case		Control		Crude OR	95% CI	*p-value*
	n	%	n	%			
Stenotrophomonas maltophilia							
No	137	92.6	283	95.9	1		
Yes	11	7.4	12	4.1	1.89	0.82-4.40	0.201
E. faecium							
No	135	91.2	268	90.8	1		
Yes	13	8.8	27	9.2	0.96	0.48-1.91	1.00
Others microorganisms							
No	126	85.1	265	89.8	1		
Yes	22	14.9	30	10.2	1.54	0.86-2.78	0.196

OR = Odd Ratio
*statistical significane at $\alpha < 0.05$

5. Factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection by multiple logistic regression.

The factors considered to be significantly associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection in univariated analysis were co-morbidity, malignancy, chronic renal failure, duration of admission prior to infection > 1 week, treat dexamethasone, invasive procedure, mechanical ventilation, retained urinary chatheter, central venous line, nasogastric intubation, vancomycin, carbapenem, ward admission, microorganisms isolated, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis*.

This association might be influenced by confounding factors. In order to exclude the potential confounder, unconditional multiple logistic regression was analyzed, identifind 3 factors significant of associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection; normally malignancy, chronic renal failure and nasogastric intubation (Table 9).

Co-morbidity

Univariate analysis found the association between co-morbidity and cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. By multiple logistic regression analysis, co-morbidity was not found to be associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (adjust OR = 1.14, 95%CI= 0.65-2.00). However, both univariate and multiple logistic regression analysis found the association between malignancy and cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (OR = 3.00, 95%CI = 1.48-6.10). Furthermore, univariate and multiple logistic regression analysis found the association between chronic renal failure and cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (adjust OR= 2.90, 95%CI = 1.39-6.05) (Table 9).

Duration of admission prior to infection

By univariate analysis, there was association between patients who had long duration of admission prior to infection with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. By multivariate analysis, the duration of admission prior to infection was not found to be associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (adjust OR= 1.513, 95%CI= 0.666-3.438) (Table 9).

Immunosuppressive treatment

By Univariate analysis, found that patients who received dexamethasone treatment had a higher risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. But by multivariate analysis there were associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection, no statistical significant (OR= 1.18, 95%CI =0.44-3.16) (Table 9).

Invasive procedure

Univariate analysis showed the association between invasive procedures and cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection, but by multivariate analysis, they were not associated with cefoperazone/sulbactam-resistant *A.baumannii* nosocomial infection (adjust OR=0.497, 95%CI= 0.168-1.469)(Table 9).

The result by univariate concern associated between patients who on mechanical ventilation with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection found that on mechanical ventilation ≤ 1week and > 1 week increased the risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. By multivariate analysis, mechanical ventilation ≤ 1week and > 1 week were not associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection(adjust OR= 0.996, 95%CI= 0.522-1.898 and adjust OR= 1.367, 95%CI= 0.730-2.557), respectively (Table 9).

Furthermore, univariate analysis identified the associated between patients retained urinary catheter > 1week with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection, also by multivariated analysis demonstrated the association (adjust OR= 1.17,95%CI = 0.60-2.29), but no statistical significance compared with patients those without urinary catheter (Table 9).

Univariated and multivariate analysis identified the association between central venous line and cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. By multivariate analysis increased the risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection but no statistical significant, OR for on central venous line ≤ 1 week was 1.89(95%CI= 0.98-3.66) and on central venous line > 1 week was OR 1.50 (95%CI=0.64-3.48) compared with who those without central venous line (Table 9).

Univariated and multivariate analysis demonstrated the associated between nasogastric intubation and cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. By multivariate analysis, the patients on nasogastric intubation ≤ 1week had increased risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection(adjust OR= 2.13, 95%CI= 1.13-4.01), and patients who on nasogastric intubation > 1 week (adjust OR= 2.38,95%CI=1.21-4.70) compared with who those without nasogastric intubation (Table 9).

Antimicrobial agents

Univariate analysis found the association between antimicrobial agents with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection; vancomycin and carbapenem. By multivariate analysis, vancomycin use ≤ 1week and

> 1 week were not found to be associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (adjust OR=1.84,95%CI=0.77-4.41 and adjust OR= 1.46, 95%CI=0.62-3.42), respectively, also carbapenem use (adjust OR= 1.15, 95%CI=0.60-2.20 and adjust OR= 1.01, 95%CI= 0.50-2.02), respectively (Table 9).

Ward admission

Univariate analysis found the ward admission associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection, especially ICU ward (adjust OR= 1.78, 95%CI=0.99-3.19) which had higher risk more than other ward. The surgery ward was adjust OR 1.10 (95%CI=0.61-2.00 compared with general ward (Table 9).

Microorganisms isolated

Univariate analysis found the associated between microorganisms isolated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. By multivariate analysis, microorganisms isolated was not associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection (adjust OR= 1.29, 95%CI= 0.74-2.26) also *Pseudomonas aeruginosa* (adjust OR= 1.26,95%CI= 0.69-2.31), *Escherichia coli* (adjust OR=1.27, 95%CI = 0.71-2.27) and *Enterococcus faecalis* (adjust OR=2.48, 95%CI =0.86-7.16) (Table 9).

Table 9 Factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection by multiple logistic regression.

Risk factors	Crude OR	95% CI	Ajusted OR	95% CI	*p-value*
Patient factors					
Comorbidity	1.95	1.21-3.13	1.14	0.65-2.00	0.638
Malignancy	2.78	1.51-5.14	3.00	1.48-6.10	0.002*
Chronic renal_failure	2.53	1.36-4.71	2.90	1.39-6.05	0.005*
Duration of admission prior to infection					
> 1 weeks	1.70	1.14-2.54	1.51	0.67-3.44	0.323
Treatment factors					
Dexamethasone	1.62	1.04-2.51	1.18	0.44-3.16	0.747
Invasive procedure	2.45	1.06-5.70	0.50	0.17-1.47	0.206
Retained urinary catheter					
≤ 1 weeks	1.45	0.86-2.45	1.35	0.72-2.53	0.358
> 1 weeks	2.75	1.72-4.40	1.17	0.60-2.29	0.652
Central venous line					
≤ 1 weeks	1.30	0.72-2.33	1.89	0.98-3.66	0.058
> 1 weeks	3.10	1.85-5.06	1.50	0.64-3.48	0.349
Mechanical ventilation					
≤ 1 weeks	1.91	1.18-3.10	1.00	0.52-1.90	0.989
> 1 weeks	2.51	1.54-4.07	1.37	0.73-2.56	0.329
Nasogastric intubation					
≤ 1 weeks	2.13	1.25-3.62	2.13	1.13-4.01	0.019*
> 1 weeks	3.51	2.20-5.98	2.38	1.21-4.70	0.012*
Antimicrobial agent					
Vancomycin					
≤ 1 weeks	2.84	1.29-6.26	1.84	0.77-4.41	0.170
> 1 weeks	2.14	1.05-4.38	1.46	0.62-3.42	0.383
Carbapenem					
≤ 1 weeks	1.62	0.93-2.84	1.15	0.60-2.20	0.676
> 1 weeks	2.24	1.27-3.95	1.01	0.50-2.02	0.983
Environment factors					
Ward admission					
Surgery	1.11	0.66-1.87	1.10	0.61-2.00	0.754
ICU	2.02	1.22-3.35	1.78	0.99-3.19	0.052

Crud OR = Crud Odd Ratio
Adjust OR = Adjusted Odd Ratio, adjust by multivariate technique
*statistical significane at $\alpha < 0.05$

Table 9 Factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection by multiple logistic regression. (cont.)

Risk factors	**Crude OR**	**95% CI**	**Ajusted OR**	**95% CI**	***p-value***
Microorganisms isolated with *A. baumannii*	2.23	1.46-3.42	1.29	0.74-2.26	0.373
P. aeruginosa	1.972	1.23-3.17	1.26	0.69-2.31	0.454
E. coli	2.260	1.44-3.56	1.27	0.71-2.27	0.422
E. faecalis	3.165	1.27-7.92	2.48	0.86-7.16	0.092

Crud OR = Crud Odd Ratio
Adjust OR = Adjusted Odd Ratio, adjust by multivariate technique
*statistical significane at $\alpha < 0.05$

CHAPTER V
DISCUSSION

Data collection in study to were collected by researcher to decrease information bias. Multicollinearity was appeared in this study because one patient received many factors such as ; patient who had diabetes mellitus with chronic renal failure or diabetes mellitus with hypertension, who had invasive procedure more than one type at the same time but this problem was solved by multivariated analysis.

1. Incidence of cefoperazone/sulbactam-resistant

The incidence of cefoperazone/sulbactam-resistant *A. baumannii* infection during January to November, 2007 was 6.2 and the occurrence during the March, February and June were higher than other month of the year (10.1%,8.6% and 8.0%), respectively. While Abbo A. *et al* (58) found that the occurrence of multidrug-resistant *A. baumannii* nosocomial infection had higher level during January, March and April. Our result were difference the due to number of admitted patients in each month of year in Rajavithi Hospital were not the same as the other study. The incidence of cefoperazone/sulbactam-resistant *A. baumannii* in this study showed lower, so in Rajavithi Hospital can use cefoperazone/sulbactam treat *A. baumannii* nosocomial infection to effectively.

The case fatality rate in this study showed that, the case group (62.16/1,000 patients) was higher than the control group (42.0/1,000 patients), our result was the same as study of Know K.T. *et al* (11) they found that higher mortality rate in patients who were imipenem-resistant *A. baumannii* nosocomial infection more than who were imipenem-susceptible *A. baumannii* nosocomial infection (57.5%, 27.5%), respectively.

2. Factors associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection

2.1 Patient factors

Sex, age and duration of admission prior to infected were not were associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection. This were due to these factors were evenly distribute in case and control group, so the was to association with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections.

Co-morbidity such as malignancy and chronic renal failure were associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection, these due the malignancy in long term can impair the immunity of the patients, chronic renal failure patients always had severe clinical. The most variable of co-morbidity such as diabetes mellitus, HIV, neurogical disease, animia, heart disease, liver disease, lung disease, hypertension, old CVA not associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection, these may be due to most patients in our study were elderly , so the elderly patients always had from the diseases and chronic diseases both in case and control group.

2.2 Treatment factors

Immunosuppressive treatments such as: dexamethasone, prednisolone, chemotherapy were not associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection those may be due to the number of the patients who had received immunosuppressive treatment were less number so it did not showed any relation to cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections.

Central venous line was not associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection due to had our study showed that the events were similar in both case and control group, so it did not showed association sifnificantly.

Nasogastric intubation $\leq$ 1 week and > 1 week increased risk of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection which were the same as many studies before (14),(61),(59). Infections related to long term nasogastric intubation were more often due to the pathogens could access the respiratory tract via

the nasogastric tubes and the use of nasogastric for feeding related with aspiration. Infection related to catheters came from the microorganisms colonized from multiple sources, most often by microorganisms that colonize the skin surrounding the insertion site (81).

Most other variables of invasive procedures factors such as central venouse line, urinary catheter, mechanical ventilator and other invasive procedures were significant risk factors by univariate analysis but were not significant by multivariate analysis. These maybe due to most variables had multicollinearity and had low incidence of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections.

Antimicrobial agents, by univariate analysis presented that carbapenem use > 1 week was statistical significant risk factors for cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections these was similar to the other studies (78),(62),(71). But by multivariate analysis, it was not significant may be due to multicollinearity with several antimicrobials or had low incidence of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections.

Univariate analysis that using vancomycin ≤ 1 week and > 1 week associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection, which was the same as the other reports (62). Infections related to long-term vancomycin use was more often, but presented by multivariate analysis was not associated maybe due to multicolinearity or had low incidence of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection.

Moreover, univariate analysis showed the use of penicillin was the protect about cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections, also the same as the report of Abbo A. *et al* (58) but no statistical significant.

The other variables of antimicrobial drug uses such as : quinolone, aminoglycoside, cephalosporin 1^{st}- 4^{th}, macrorolide, sulfonamide, metronidazole and fosfomycin were not associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection these may be due to variouse antimicrobial treatment previously had drugs sensitivity test, or time of antimicrobial use not proper and multicoliinearity of antimicrobial drug includ had low incidence of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection.

2.3 Environment factors

Univariate analysis presented the association of ward admission with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections but by multivariate analysis it was not statisticaly significant, due to we had less number of population or selection bias of ward type in our study because in Rajavithi Hospital classification of ward type was different from the other hospital and we had the low incidence of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection in ICU ward.

2.4 Co- isolated microorganisms

Pseudomonas aeruginosa, *Escherichia coli*, *Enterococcus faecalis* presented the association with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection by univariate analysis, also but were not statistical significantly by multivariate analysis. This may be due to we had co-isolation which were similar in both case and control group.

CHAPTER VI
CONCLUSION AND RECOMMENDATION

The results from univariate analysis, the factors which found to be significantly associated with cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections were co-morbidity , malignancy, chronic renal failure, duration of admission prior to infection, treat dexamethasone, invasive procedures, retained urinary catheter, central venous line, mechanical ventilator, nasogastric intubation, vancomycin use, carbepenem use, ward admission, co-isolated, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis.*

But when these factors were adjusted for the possible confounders, only 3 factors were found to be significantly, malignancy (OR= 3.00, 95%CI=1.48-6.10), chronic renal failure (OR= 2.90, 95%CI= 1.39-6.05), on nasogastric intubation $\leq$ 1 week (OR= 2.13, 95%CI= 1.13- 4.01), on nasogastric intubation > 1 week (OR= 2.38, 95%CI= 1.21 - 4.70)

In conclusion, cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection in this study was associated with the underlying disease and invasive procedure.

Recommendation for implication of the results

To decrease the risk factors of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infections, it was recommended that.

1. We recommended that be careful about patient who had co-morbidity, especially malignancy and chronic renal failure became these were risk factors infection.

2. Invasive procedure, especially nasogastric intubation should be applied only when necessary and for only the shorten period possible.

3. Oral hygiene might reduce the incidence of cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection because our results showed that most specimens were sputum. Sputum was related with lower respiratory tract infection, The other study support oral hygiene reduce VAP.

4. The results of this study got data from teaching hospital, so it had some different factors that were not the same as general hospitals (environment, department, ward, prevalence of *A. baumannii* nosocomial infection, severity of diseases in patients etc.). Therefore, in case of using this results for comparing with general hospital should be careful consider.

Recommendations for the further study

1. The further study should be the Minimal Inhibitory Concentration (MIC) of cefoperazone/sulbactam for reliability of data and can define case and control clealy.

2. Further study should be research about cost burden of hospital playment cefoperazone/sulbactam-resistant *A. baumannii* nosocomial infection compare cost of cefoperazone/sulbactam-suceptible *A. baumannii* nosocomial infection because of long term admission and higher cost antimicrobial drug treat.

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APPENDIX

Data collection form

ID……HN……
( ) Case ( ) Control

Part 1 General characteristics

1.1 Age……Years

1.2 Gender ( ) Male ( ) Female

1.3 Occupation…….

1.4 Principle diagnosis……..

1.5 Admission date ……/……/…… Discharge date……/……/…….
Length of hospital…….day

1.6 Discharge status
( ) Dead ( ) Complet recovery ( ) Improve ( ) Not improved

1.7 Patent
( ) UC ( ) SC ( ) CS ( ) Payment

Part 2 *Acinetobacter baumannii* nosocomial infection

2.1 Site of infection
( ) Lower respiratory tract
( ) Urinary
( ) Surgical wound
( ) Blood stream
( ) Skin & Soft tissue
( ) Gastrointestinal
( ) Eye & Ear
( ) Others……

2.2 Specimens
( ) Sputum
( ) Wound pus
( ) Urine
( ) Blood
( ) Pleural fluid
( ) Peritoneal fluid
( ) Cerebrospinal fluid
( ) Other……...

Part 3 Risk factors

3.1 Admission date ……/……/……Sent specimens date……/……/……

Length of *A.baumannii* Positive …….day

3.2 Ward admission

Medicine

( ) ICU Medicine ( ) CCU ( ) Medicine (EMS 3 floor)

( ) Medicine (male) ( )Medicine (female) ( ) Medicine (Floor 6)

( ) Medicine (Private 4 beds room) ( ) Medicine (Single bed room)

General Surgery

( ) Surgery Male ( ) Surgery female ( ) Surgery Private 4 beds room)

( ) Surgery (Single bed room) ( ) Surgery ICU

Neurosurgery

( ) Neurosurgery general ward ( ) ICU Neurosurgery

Orthopedic

( ) Orthopaedic Male ( ) Orthopedic female ( ) Orthopedic private

CVT

( ) CVT general ward ( ) ICU CVT

ENT

( ) Ward ENT

OB-GYN

( ) General female

( ) Single bed

( ) 4 Bed Room

Other……..

3.3 Transfer from the Other ( ) No ( ) Yes

Medicine

( ) ICU Medicine ( ) CCU ( ) Medicine (EMS 3 floor)

( ) Medicine (male) ( )Medicine (female) ( ) Medicine (Floor 6)

( ) Medicine (Private 4 beds room) ( ) Medicine (Single bed room)

General Surgery

( ) Surgery Male( ) Surgery female ( ) Surgery Private 4 beds room)

( ) Surgery (Single bed room) ( ) Surgery ICU

Neurosurgery

( ) Neurosurgery general ward ( ) ICU Neurosurgery

Orthopedic

( ) Orthopaedic Male ( ) Orthopedic female ( ) Orthopedic private

CVT

( ) CVT general ward ( ) ICU CVT

ENT

( ) Ward ENT

OB-GYN

( ) General female ( ) Single bed

( ) 4 Bed Room

Other……..

3.4 Co-morbidity

( ) No

( ) Yes

( ) Diabetes Mellitus ( ) Renal failure
( ) HIV ( ) Heart disease
( ) Malignancy ( ) Liver disease
( ) Neurological disease ( ) Lung disease
( ) Anemia ( )
Other……..

3.5 Immunosuppressive treatment ( ) NO ( ) Yes

( ) Steroid (specify)……
( ) Chemotherapy
( ) Radiotherapy

3.6 Invasive procedure ( ) No ( ) Yes

	Start	*A.baumannii* Positive	Total(day)
( ) Urinary catheter	……/…../…….	……../……/……	total……day
( ) Mechanical Vetilation	……/…../…….	……./……./……	total…….day
( ) Central line	……/…../…….	..……/……./……	total…….day
( ) NG tube	……/…../……..	……/……./…….	total…….day
( ) Other(specify) ……	….. /…../……..	……/……./…….	total…….day

3.7 Surgery ( ) No ( ) Yes

3.8 Antimicrobial drug use ( ) No ( ) Yes

	Start	*A.baumannii* Positive	Total(day)
( ) Ampicilline	……/……/……..	……./……./……	……
( ) Augmentin	……/……/……..	……./……./……	……
( ) Cephalotin	……/……/……..	……./……./……	……
( ) Tienam	……./……/……..	……./……./……	……
( ) Meropenem	……../……/……..	……./……./……	……
( ) Ceftazidime	..…../……/……..	……./……./……	……...
( ) Ceftriaxone	……/……/……..	……./……./……	……
( ) Amikacin	……/……/……..	……./……./……	……
( ) Gentamycin	……/……/……..	……./……./……	……
( ) Netromycine	…../……/……..	……./……./……	……
( ) Ciprofloxacin	..…../……/…….	……./……./……	……
( ) Levofloxacin	…./……/……. .	..…../……./……	……
( ) Sulperazone	……/……/……..	.. ../……./……	……
( ) Cotrimoxazole	..…/……/……..	...…./……./……	……
( ) Metronidozole	…./……/…….	……./……./……	……
( ) Other	...../……/…….	……./……./……	……
( ) Other	...../……/…….	……./……./……	……
( ) Other	...../……/…….	……./……./……	……

3.9 Co- isolated ( ) No
( ) Yes

( ) *Pseudomonas aeruginosa*
( ) *Escherichia coli*
( ) *Proteus spp.*
( ) *Enterobacter spp.*
( ) *Staphylococcus aureus*
( ) *Klebsiella spp.*
( ) Other (specify).....
( ) Other (specify).....

BIOGRAPHY

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