旅行に対する適切な感染危険情報も少ない。このような状況を鑑み、本研究では、東南アジア地域での本症の実態把握、流行状況調査、診断能力の向上ならびに分離されたヒストプラスマ属の各国での疫学的解析や基礎研究の推進・発展主な内容として、それぞれの国の感染症研究機関との共同研究ネットワークを構築し、日本ならびにアジア諸国の公衆衛生に貢献することを目的とする。

B. 研究方法

ネットワーク構築対象のアジアの感染症研究機関として、タイ王国の国立衛生研究所(The National Institute of Health : NIH, Thailand)、チェンマイ大学（医学部微生物学講座）ならびにベトナム社会主義共和国の国立衛生疫学研究所（National Institute of Hygiene and Epidemiology: NIHE, Vietnam）を選定し、各国におけるヒストプラスマ症に対する認識、見解について意見交換を行い、ヒストプラスマ症に対する共同研究やラボラトリーネットワークについて検討を行った（図1）。

共同研究に際しては、本年度はタイ、ベトナムにおけるヒストプラスマ症の実態調査、生息状況に関する調査、環境リスク因子の同定から開始することとした。生息調査に関しては、環境検体（土壌）の培養法、遺伝子検出法を用いた。

1）培養法

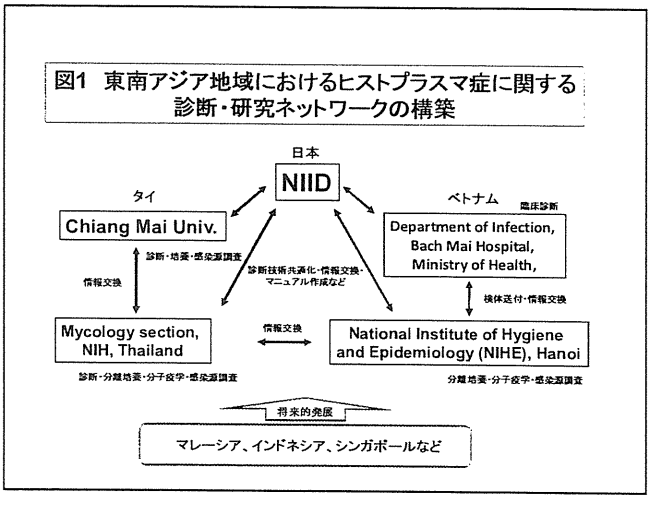
ヒストプラスマ属が存在すると考えられるコウモリや鳥類の糞で汚染された土壌を採取し、クロラムフェニコール含有PBSに懸濁した後振盪攪拌し、1-3時間静置した上清を一部採取しBHI培地へ塗布し、30℃で8週間培養した。

2）遺伝子検出法

対象検体は培養法に供した検体とし、PBSに懸濁した上清をproteinase K処理、β-グルカナーゼ処理し、フェノール・クロロフォルム法でDNA抽出した。遺伝子検出には我々が用いているヒストプラスマ属のM antigenを標的とするnested PCR法を適用し、プライマーはすでに報告した（Ohno H et al. Internal Medicine, 49, 2010) Msp1F、Msp2Rをfirst PCRで、Msp2F、Msp3Rをsecond PCRで用い、反応条件も同様に準じた。

（倫理面からの配慮について）

環境検体採取に関しては、研究目的、内容を土地所有者等に説明し許可を得て採取した。




C. 研究結果

1）タイ国、ベトナム国におけるヒストプラスマ症の現状

タイ国でのヒストプラスマ症はわが国と違い、バンコク市やチェンマイ市でも比較的多く患者発生は認められ、なかでもHIV感染者に合

併する日和見感染症として播種型も認められるとの事であった（タイ全土で 1984 年から 2009 年で 1,001 例）。またチェンマイ大学病院におけるヒストプラスマ症患者は、2000 年から 2010 年までで、培養陽性で診断が確定した症例が 36 例、培養陽性検体では骨髄やリンパ節が多いことから、播種型が多いことが伺われた。同国におけるヒストプラスマ症における問題点は、１）培養陽性率が低い、２）感染源、危険因子が同定できていない、３）結核との鑑別が困難な例が散見される、などであった。また、過去にタイ国内の土壌サンプルを使用し、ヒストプラスマ属の分離を試みた研究はあるが、未だ土壌からヒストプラスマ属の分離に成功した例はないとの事であった。

一方、ベトナム国では今回はハノイ市のある北部地方についての状況について意見交換を行った（Dr. Nguyen Van Tien, Bach Mai Hospital, Hanoi らを交え）。ベトナム北部では近年の HIV 感染者の増加を受けて、これらに合併するマルネッフェイ型ペニシリウム症やクリプトコックス症などの日和見真菌症の頻度が高いとの事であったが、ヒストプラスマ症については統計は取っていないものの稀ではない真菌症であるとのことであった。この地方ではヒストプラスマ症の診断は臨床的診断が主で、検査法として塗抹法は行っているが、培養法は行っていないこと、遺伝子診断法は導入していないという状況であった。しかし、少なくとも北部ベトナムでも迅速診断法や、感染源、リザーバーとなりうるものの同定、感染対策の重要性は理解されていた。

以上のような状況から、国立感染症研究所とタイ NIH、チェンマイ大学、ベトナム NIHE とのヒストプラスマ症に関する疫学をはじめとする基礎研究や、診断技術、治療能力の向上をめざした共同研究を継続して行うことで合意ができた。

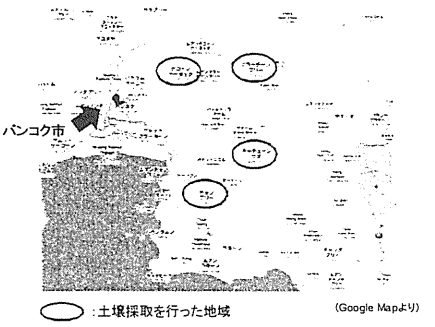
２）バンコク近郊でのヒストプラスマ属生息調査

本年度は、タイ NIH の真菌研究室のスタッフとともに、タイ・バンコク近郊の 4 県（図 2）を対象に、おもに公共の場所（寺院境内など）を中心にコウモリの糞で汚染された土壌を採取し、ヒストプラスマ属の培養、遺伝子検出を行った。土壌検体は計 41 検体採取し、培養結果はいずれも陰性であったが、23 検体（56%）でヒストプラスマ検出用 PCR が陽性であった（図 3）。現在、陽性検体については塩基配列の確認を行っている。

３）ハノイ近郊でのヒストプラスマ属生息調査

本年度はバンコク近郊で行ったのと同様な土壌検体を対象としてサンプリングを行った（Dr. Kieu Anh, NIHE らによる）。現在、培養法、遺伝子検出の検討を行っている。

図2 環境検体の採取地域（タイ・バンコク近郊）



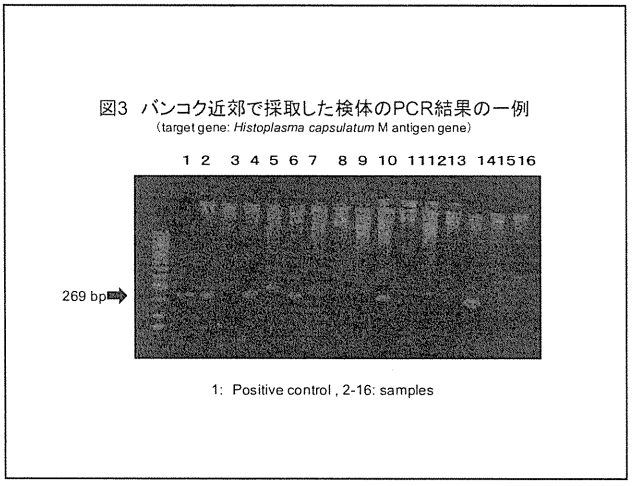


図3 バンコク近郊で採取した検体のPCR結果の一例
(target gene: *Histoplasma capsulatum* M antigen gene)

D. 考察

ヒストプラスマ症は、わが国では海外で感染し国内で発病する、いわゆる輸入真菌症とされているが、東南アジア、とくにタイ、ベトナムでは HIV 感染者を中心に比較的高い頻度で認められる真菌症である。本感染症は基本的にヒトーヒト感染がないことや、診断が困難であることから、これらの国では疫学情報の不足が認められる。日本人患者の多くは東南アジアでの感染者であることが考えられているが、このことも感染危険因子、ハイリスクな自然環境などの情報、エビデンス不足が原因となっていることは否めない。

今回われわれは上記のような背景から、タイ、ベトナム両国の感染症担当研究機関と接触を行い、ヒストプラスマ症に対する総合的な対策の一環として、基礎的、臨床的共同研究の提案を行い、合意を得ることに至った。両国とも感染源、リザーバーの同定はほとんど検討されておらず、今回タイ国での公共利用の土地の土壌検体を中心にヒストプラスマ属の存在を検証した。その結果、生菌は証明できなかったものの、多くの検体で菌遺伝子の存在が疑われた。現在、最終確認を行っているが、これらがヒストプラスマ属の遺伝子であるならば、バンコク近郊においてヒストプラスマ属は特殊な環境ではなく、日常的に人間が生活する空間に生息していることが推測され、感染源、感染経路解明、感染対策の一助となる可能性がある。これに関して今後同様に土壌中に生息するクリプトコックス属などの生息状況も検討することで、相対的な病原性、感染危険度なども検討していきたいと考えている。

一方、ベトナム国においては今回の共同研究提案でヒストプラスマ症が再認識された点もあり、共同での生息状況調査がようやく開始されたばかりで、現在試料の解析中である。今後これらの結果を見ながら、更なる検討を重ねていく予定である。

E. 結論

タイ・バンコク近郊において複数の公共地点の土壌からヒストプラスマ属の遺伝子と考えられる遺伝子が検出されたが、真の感染源となりうるのか、発病の危険性等については更なる検討が必要である。

F. 健康危険情報

なし

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H.　知的財産権の出願・登録状況

１．　特許取得

なし

２．　実用新案登録

なし

３．　その他

なし

MINISTRY OF HEALTH
NATIONAL INSTITUTE OF HYGIENE AND EPIDEMIOLOGY
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**The FIRST PHASE REPORT**

# STRENTHENING THE RESEARCH CAPACITIES OF THE NATIONAL INSTITUTE OF HYGIENE AND EPIDEMIOLOGY ON SOME NEGLECTED INFECTIOUS DISEASES IN VIETNAM

*(Sponsored by*
*the National Institute of Infectious Diseases, Tokyo, Japan)*

**Hanoi, March 2012**

## THE FIRST PHASE REPORT

1. **Project title:**

- Strenthening the research capacities of the National Institute of Hygiene and Epidemiology on some neglected infectious diseases in Vietnam.

2. **Host agency:**

- Ministry of Health, Vietnam

3. **Implementation agency:**

- National Institute of Hygiene and Epidemiology, Vietnam

4. **Research sponsor**

- National Institute of Infectious Diseases, Japan.

5. **Research titles**

5.1. **Research 1:** Molecular epidemiology, toxin profile and antibiotic resistance of Clostridium difficile infection in some hospitals in the North of Vietnam.

5.2. **Research 2:** Clinical epidemiology and molecular characterization of Enterobacteriaceae strains producing Metallo-Beta-Lactamase (including NDM-1) in some hospitals in Hanoi city.

5.3. **Research 3:** Molecular epidemiologic analysis of V. cholerae O1 isolates in Vietnam from 2007 to 2009.

5.4. **Research 4:** The basic and clinical study on Histoplasmosis in Vietnam.

5.5. **Research 5:** Establishment of laboratory diagnosis for leptospirosis and investigation of prevalence of leptospirosis among patients with fever of unknown origin in northern area of Vietnam.

5.6. **Research 6:** The improvement of the epidemiological surveillance of anthrax in Vietnam.

5.7. **Research 7:** Enhancement of the National Institute of Hygiene and Epidemiology rabies laboratory capacity for rabies/bat lyssavirus diagnosis and research

5.8. **Research 8:** Phylogenetic analysis and transmission dynamics of measles and rubella viruses isolated from some outbreaks in the Northern provinces of Vietnam from 2006 to 2014

5.9. **Research 9:** Epidemiology and molecular characteristics of the hand, foot and mouth disease in the North of Vietnam.

6. **Research duration:**

- 3 year (2012-2014)

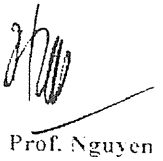
7. **Study budget:**

- Total funding for the project is 36.000.000 JPY for 36 months from 2012-2014
- Funding status: National Institute of Hygiene and Epidemiology, Vietnam has received the fund for the first phase which was of 7.000.000 JPY.

8. **Implementation activities**

- In attached sheets

Hanoi, 15 March, 2012
Signature



Assoc. Prof. Nguyen Tran Hien, MD., MPH., PhD.
Director
National Institute of Hygiene and Epidemiology

**Research 1**

**1.1 Project title: Molecular epidemiology, toxin profile and antibiotic resistance of Clostridium difficile infection in some hospitals in the North of Vietnam.**

**1.2 Objectives**

**1st stage**:

- To set up a laboratory for anaerobic bacteria research: prepare necessary materials and reagents for anaerobic bacteria culture and other bacterial detection method
- To establish a SOP for etiologic diagnosis of *Clostridium difficile* infection in Vietnam (bacterial culture and/or toxin detection).
- To investigate baseline information on antibiotics-associated diarrhea in hospitals in order to select study sites for sample collection.

**2nd stage**:

- To estimate the proportion of cases of Clostridium difficile infection among hospitalized patients presenting with antibiotics-associated diarrhoea (AAD) or colitis in some major hospitals in Northern Vietnam.
- To determine risk factors of Clostridium difficile infection and its severity in Vietnam, especially geriatric population

**3rd stage**:

- To study on molecular epidemiology of Clostridium difficile isolates in Vietnam
- To explore toxin profile and antibiotic resistance of Clostridium difficile isolates

**1.3 Name of Researchers**
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[2] National Institute of Infectious Diseases, Tokyo, Japan

**1.4 Affiliation**
- National Institute of Hygiene and Epidemiology, Vietnam.
- National Institute of Infectious Diseases, Japan.

**1.5 Sub-project title:** First study on *Clostridium difficile* and its infection in Vietnamese hospitals: prevalence, risk factors, molecular epidemiology, toxin profile and antibiotic resistance.

**1.6 Summary**
*Clostridium difficile* causes a range of illness from mild diarrhea to pseudo membranous colitis and death. *C. difficile* colonizes the human intestinal tract via the fecal-oral route. This colonization is facilitated by disruption of normal intestinal flora due to antimicrobial therapy. *C. difficile* infection is one of the most common healthcare-associated infections and a significant cause of morbidity and mortality among elderly hospitalized patients. Besides its occurrence in the hospital, the infection has also become more common in the community.

Recently, *C. difficile* infections have become more frequent, more severe, more difficult to treat, and more likely to recur. In the United States, *C. difficile* infections affect more than 60 hospitalized patients per 100,000 (0.06 percent). In Asia, *C. difficile* has been notified as a emerging and re-emerging pathogen in Japan, China, Singapore and Thailand (Wongwanich, Rugdeekha et al. 2003; Kato, Ito et al. 2007; Huang, Wu et al. 2009; Lim, Ling et al. 2011). These observations have been attributed to a new strain designated BI, NAP1 or ribotype 027. This strain appears to be more virulent than other strains, which may be attributable to increased toxin production compared to conventional strains. Fluoroquinolone use has strongly correlated with the emergence of this strain.

*C. difficile* infection surveillances have been established and become mandatory in many countries in the world such as in the US, UK, Ireland and Japan. However, *C. difficile* infections have not ever been reported in Vietnam possibly due to lack of surveillance system and laboratory support. Therefore, this project will be the first study on *C. difficile* and *C. difficile* infection in Vietnam. The study results

possibly highlight that *C. difficile* infection is an important issue of public health in Vietnamese hospitals and provide evidence to establish a program for prevention and infection control of *C. difficile* infection.

### 1.7 Purposes

Research on anaerobic bacteria in Vietnam has been ignored for many years. Literature survey has showed that only two studies on anaerobic bacteria of *Bactericides fragilis* and *Clostridium perfringens* have been found from publications of Vietnam. However, study on *C. difficile* infection has never been done in Vietnam. There are possible reasons to explain this situation. They include:

a. **Culture of *C. difficile* is hampered by technical and costly limitations**: *C. difficile* is an obligate anaerobic bacterium that requires special media and strict conditions to growth. Thus culturing these bacteria is very difficult, expensive, laborious and time-consuming.
b. **The optimal approach for laboratory diagnosis of *C. difficile* is uncertain:** There are two categories of laboratory tests for *C. difficile:* toxin assays (which evaluate for evidence of toxin) and organism detection assays (which evaluate for the presence of organism). Anaerobic stool culture for isolation of *C. difficile* is the most sensitive test but is not practical due to its slow turnaround time; the need to detect toxin production by the recovered isolate further slows down this approach. Cytotoxicity assay also takes too long for routine clinical use. Enzyme immunoassay testing for *C. difficile* toxins A and B is rapid but less sensitive. Polymerase chain reaction (PCR) of stool appears to be rapid, sensitive and specific, but need more data to support routine use of this modality.
c. **No previous data and report on *C. difficile* infection is available in Vietnam**. It therefore attracts limited attention of clinicians and may underestimate the importance of this infection.

Laboratory of pathogenic anaerobes of National Institute of Hygiene and Epidemiology, Vietnam has newly established since 20th September 2011. The goal of this laboratory is to establish and promote research on anaerobic pathogens. Being aware of the importance of *C. difficile* and its potential role in severe antibiotics-associated diarrhea cases reported from hospitals in Vietnam, we want to launch a first study on anaerobic *C. difficile* infection in Hanoi. This study will not provide a first estimate of the proportion of the cases of antibiotics-associated diarrhea that are due to *Clostridium difficile*, also pave a way for another researches on anaerobic bacteria in Vietnam.
The objectives of this 3-year project will be divided into 3 stages:

**1st stage**:

a. To set up a laboratory for anaerobic bacteria research: prepare necessary materials and reagents for anaerobic bacteria culture and other bacterial detection method
b. To establish a SOP for etiologic diagnosis of *Clostridium difficile* infection in Vietnam (bacterial culture and/or toxin detection).
c. To investigate baseline information on antibiotics-associated diarrhea in hospitals in order to select study sites for sample collection.

### 1.8 Methods

a. To set up a laboratory for anaerobic bacteria research: do literature survey to learn about detection techniques of *Clostridium difficile*. From basic knowledge of *Clostridium difficile* detection, order and prepare reagents, media, commercial kits and primer sequences for molecular assays to detect *Clostridium difficile* and toxin A and B directly from stool samples or bacterial colony.
b. To establish a SOP for etiologic diagnosis of *Clostridium difficile* infection: Collect information from literature together with consultancy from anaerobic experts to establish a protocol for laboratory-based diagnosis of *Clostridium difficile* infection in Vietnam.
c. To investigate baseline information on antibiotics-associated diarrhea in hospitals: interviews with clinicians in hospitals together with retrospective review of medical records in intensive care units of hospitals in Hanoi to approximately estimate rough proportion of the disease in the hospitals.

## 1.9 Results

a. **Set up a laboratory for anaerobic bacteria research**

- Necessary materials and reagents for culturing of *Clostridium difficile* has been prepared. They include:
  - ✓ Special media for anaerobic bacteria isolation and growth (baseline media such as Brucella HK agar, GAM modified agar, GAM modified broth and specials supplements such as Proteose peptone No 2, Mannitol, Neutral red , Sodium Taurocholate , D-Cycloserine, Cefoxitin ).
  - ✓ Materials for incubation atmosphere (anaerobic pouch, anaerobic indicator for monitoring the adequacy of incubation atmosphere)
  - ✓ Incubation jars or anaerobic bag for maintenance of the atmosphere during incubation time.
- Necessary reagents for rapid detection of *Clostridium difficile* antigen: Difficile test kit and Premier Toxins A and B microwell EIA.
- Primer sequences and PCR reagents for molecular detection of *Clostridium difficile* and toxin A and B from stool samples
- International standard strains of *Clostridium difficile* for isolation and antibiotic susceptibility test have been ordering.

b. **Establish a SOP for etiologic diagnosis of *Clostridium difficile* infection**:

- A SOP for isolation, identification and toxin detection of *Clostridium difficile* has been established, starting from sample collection, sample transport and storage, sample processing, microscopy, culture, identification to toxin detection . The detail procedures have been fully described in the SOP in separate paper.

c. **Investigate baseline information on antibiotics-associated diarrhea in hospitals**

- Antibiotics-associated diarrhea occurs around 20-30 % of hospitalized patients in intensive care units of investigated hospitals. There were more frequent severe diarrhea cases with diarrhea up to more than 10 times daily and fever. Some cases were life-threatening, usually in patients with chronic diseases. No death has been reported yet.

## 1.10 Discussion

*Clostridium difficile* is an important emerging and re-emerging pathogen in the world and Asian region but this kind of infection has never explored in Vietnam. In order to understand the role of *Clostridium difficile* in antibiotics-associated diarrhea, there is essential need to establish a laboratory-based protocol for etiologic diagnosis of *Clostridium difficile* which is practical in Vietnam. This study will not only be beneficial for the establishment of a first laboratory of anaerobic bacteria research in Vietnam, also will promote further studies on other important anaerobic bacteria in the future such Clostridium botulinum, Clostridium perfringens, Bacteroids and Prevotella.

## 1.11 Publications

a. 1 publication titled "Practical laboratory approach for diagnosis of *C. difficile* among patients with antibiotics-associated diarrhea in Vietnam"

b. The paper will provide a protocol for *C. difficile* detection which can be applied in microbiology laboratory in hospitals, universities and health centres in Vietnam.

## 1.12 Reference


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### 1.13 Acknowledgements

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**Research 2**

**2.1. Project title: Clinical epidemiology and molecular characterization of Enterobacteriaceae strains producing Metallo-Beta-Lactamase (including NDM-1) in some hospitals in Hanoi city.**

**2.2. Objectives:**

a. To describe the clinical epidemiology of *Enterobacteriaceae* strains produce Metallo-Beta-Lactamase in the hospitals

b. To describe molecular characterization Metallo-Beta-Lactamase produce (including NDM-1) among *Enterobacteriaceae* strains isolated in the Hospitals of Vietnam.

**2.3. Name of Researchers**

- Dr. Tran Huy Hoang, National Institute of Hygiene and Epidemiology
- Assoc. Prof. Nguyen Binh Minh, PhD. MD., National Institute of Hygiene and Epidemiology

**2.4. Affiliation**

- National Institute of Hygiene and Epidemiology, Vietnam.
- National Institute of Infectious Diseases, Japan.

**2.1. Sub-project title:** Clinical epidemiology of Enterobacteriaceae strains producing Metallo-Beta-Lactamase (including NDM-1) in some hospitals in Hanoi city.

**2.5. Summary**

**2.6. Purposes**

One of global problem which we facing today is rapid press bacteria resistant to antibiotic through the world, especially in developing countries where have high burden of infectious diseases. The bacterial pathogenic are not only resistant to the most common antibiotics, they also resist to the last-resort antibiotic such as cephalosporin and carbapenem. The most concern recently is the spread of Gram negative bacteria which carried NDM-1 gen encodes carbapenem resistance to the several countries in Asia and Europe. The patients infected by carbapenem-resistant bacteria will increase the risk of treatment failure, costs, prolonged treatment and high mortality risk for patients. In addition, patients infected with carbapenem-resistant strains when they return home, will be a source to spread of resistant bacterial strains. With antibiotic resistance of bacteria strongly today will lead to no effective antibiotic treatment of bacteria in 5-10 years. This is a one of most global concern should be studied given appropriate measures and effective way to prevent the spread of this new resistant gene.

In Vietnam, the impact of economic reform in the early 1990's led to the explosion of the private pharmacies, the antibiotics can be easily bought without a prescription or instructions of doctors, which led to the improper use and misuse of antibiotics in Vietnam, these are important risk factors that lead to antibiotic resistance of bacteria in Vietnam:

Resistance of gram negative bacteria which are common causes of hospital infection is high. According to GARP which were reported from different studies in Ho Chi Minh city, 25% isolated were resistant to cephalosporin in 200-2001 and 42% of gram negative bacteria resistant to cefftazidime, 63% to gentamicin and 74% to nalidixic acid in 2009. The most recent study conducted by NIHE in order to determine the prevalence of carbapenem resistance strains isolated. The primary finding shown that more than 60% of *Acinetobacter* and *Pseudomonas* resistant to cephalosporin and with at least one antibiotic of carbapenem. *Enterobacteriacae* such as *E. coli* and *Klebsiella* also were resistant to cephalosporin and carbapenem at high level in some of leading hospitals of Hanoi. Therefore, the better understanding of clinical epidemiology and molecular characterization of Metallo-Beta-Lactamase producing bacteria which is causes of hospital infection in Vietnam is crucial in order to gradually put under control and drug-resistant bacteria as a basis for making treatment patients and reasonable effect.

## 2.7. Methods.

**Study design:** Cross- sectional study
**Study sites:** 3 hospitals in Hanoi (Vietduc, Sainpaul and Thanhnhan)
**Study subject:**

- Patients admitted to the hospital with all ages and genders that are being with severe infection will be selected for this study.
- *Enterobacteriacea* strains isolates from patients in the hospitals

**Sampling:** 600 (200 samples/ hospital).
**Data collection**

- **Isolation and identification:**
  - Samples will be collected from patients who have acquired infections in the hospital and then will be isolated on Macconkey agar plate. API-20 trip and specific antiserum will be used for identification of Enterobacteriaceae.
  - Antibiotic-resistant bacteria strains will be screened by Disk diffusion method
  - The minimum inhibitory concentration of antibiotic will be determined by antibiotic dilution methods in Mueller-Hinton agar, according to the guidelines of CLSI.
- **Questionnaire:** The questionnaire will be designed based on demographics information: age, gender, occupation, education level, address, date of onset, days in hospital and clinical and procedure / surgery performance, e.g. surgical methods, treatment duration, heavy, light, time of respiratory support, other issues ....The diseases together with (heart disease, diabetes, hypertension, AIDS ....) and status of antibiotic use before and during hospitalization: imipenem, meropenem, ceftriaxone, ciprofloxacin, ... are also measured.

**Variables:**

- **Demographics information:** age, gender, occupation, education level,
- History of antibiotic used before go to hospital.
- **Date:** Date of onset and days stay in hospital
- **Clinical and procedure:** surgery performance, e.g. surgical methods, medical intervention...
- **Site of isolates:** Respiratory tract, Wound, blood, urine.....
- **Other diseases:** Heart disease, diabetes, hypertension, AIDS ....)
- **Antibiotic exposure:** type of antibiotic, dose, days.....

**Data analyze:**

The data will be entered into computer and managed by Epi Data software 3.1 version and analyzed by Stata software 10.0 (Texas, US, 2007) with 5% level of significance. A frequency, percentage, average and median commands will be calculated to describe the quantitative variables.

## 2.8. Results

### a. Gram negative bacterial isolated form hospital-acquired infection patients

600 strains which caused of hospital-acquired infection were collected from three hospitals in Hanoi (Vietduc, Sainpaul and Thanhnhan) Most of strains were isolated from patient in age groups: <9, 20-29, 50-59 and highest in group of age >60 (Tab1 and Fig 1).

*Table1: Distribution of hospital infection patients by age*

| Hospital | Age groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | <9 | 10-19 | 20-29 | 30-39 | 40-49 | 50-59 | >60 |
| Vietduc | 2 | 24 | 73 | 34 | 36 | 51 | 74 |
| Sainpaul | 98 | 6 | 12 | 12 | 14 | 9 | 30 |
| Thanhnhan | 3 | 2 | 8 | 8 | 8 | 29 | 65 |
| **Total** | **103 (17.16%)** | **32 (5.33%)** | **93 (15.5%)** | **54 (9%)** | **58 (9.66%)** | **89 (14.83%)** | **169 (28.16%)** |

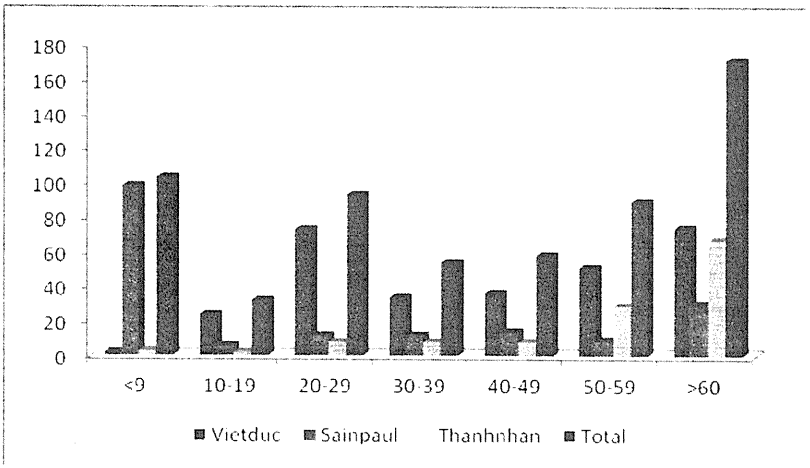


*Figure1: Distribution of hospital infection patients by age*

The Higher numbers of male than female cases 455 (75.8%) of male compared to 145 (24.2%) of female. (Tab 2 and Fig 2)

*Table 2: Distribution of hospital infection by sex*

| Hospital | Male | Femal |
|---|---|---|
| Vietduc | 246 | 48 |
| Sainpaul | 122 | 59 |
| Thanhnhan | 87 | 38 |
| **Total** | **455 (75.8%)** | **145 (24.2%)** |

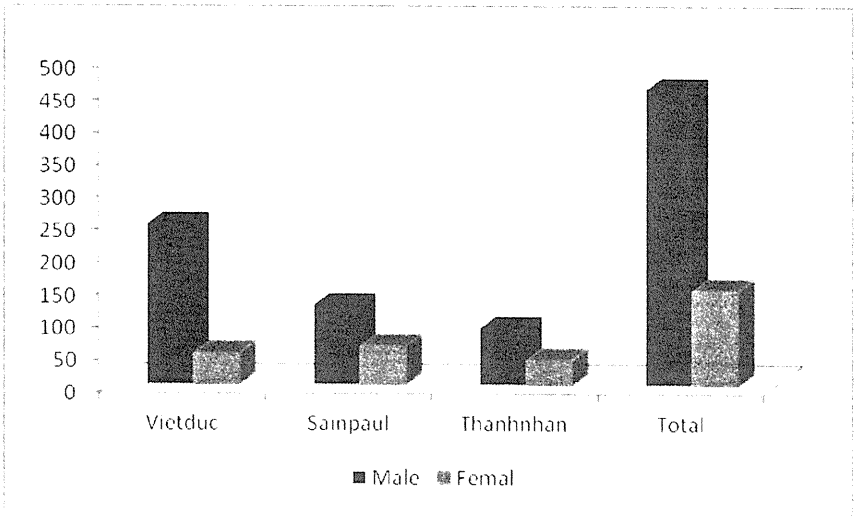


*Figure 2: Distribution of hospital infection by sex*

*Table 3: Distribution of bacterial strains isolated by hospitals*

| Hospital | *Acinetobacter* | *K. pneumonieae* | *E. cloaceae* | *E.coli* | *P.aeruginosa* | *Other Gram (-)* |
|---|---|---|---|---|---|---|
| Vietduc | 205 | 15 | 17 | 12 | 33 | 12 |
| Sainpaul | 49 | 53 | 1 | 19 | 43 | 16 |
| Thanhnhan | 66 | 26 | 1 | 6 | 9 | 17 |
| Total | **320 (53.33%)** | **94 (15.67%)** | **19 (3.17%)** | **37 (6,17%)** | **85 (14.17%)** | **45 (7.5%)** |

From three hospital (Vietduc, sainpaul and Thanhnhan) 320 (53.33%) *Acinetobacter* , 94 (15.67%) *K. pneumonieae* , 19 (3.17%) *E. cloaceae,* 85 (14.17%) *P.aeruginosa* and 45 (7.5%) other Gram negative bacterial were collected from 2011 to 3/2012 (tables 3)

***Table 4: Distribution of bacterial strains by sample collection site***

| Hospital | Collection site | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cathetere | Urine | Bronchial tube Fluid | Operation site | Blood | Sputum | Other fluids |
| **Vietduc** | 6 | 40 | 174 | 18 | 8 | 16 | 32 |
| **Sainpaul** | 0 | 0 | 72 | 9 | 16 | 5 | 79 |
| **Thanhnhan** | 1 | 4 | 1 | 2 | 1 | 41 | 75 |
| **Total** | 7 (1.16%) | 44 (7.33%) | 247 (41.16%) | 29 (4.83) | 25 (4.16%) | 62 (10.33%) | 186 (31%) |

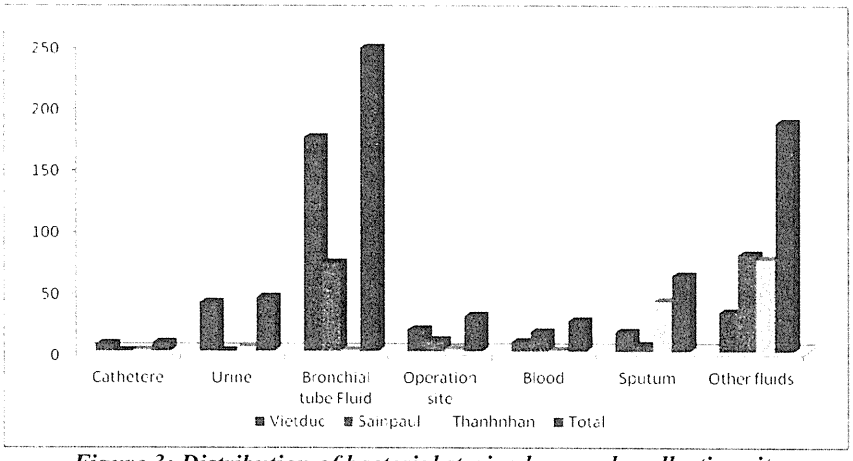


***Figure 3: Distribution of bacterial strains by sample collection site***

The highest strains were isolated from Bronchial tube Fluid 247 (41.16%), following by other fluid sample 31% (bile fluid, pancrease and abscess fluid... ), 10.33% sputum and 7.33% isolates in urine of patient (Tab 4 and Fig 3).

**b. Antibiotic sensitivity results:**

Because of the limited time we cannot perform antibiotic sensitivity testing with all of bacteria strains isolated so in this report we are going to present some primary of antibiotic results by disc diffusion method:

***Table 5: Antibiotic sensitivity testing of acinetobacter strains isolated***

| Antibiotic | Results | | |
|---|---|---|---|
| | S* | I* | R* |
| Pipera +Tazobac (n=198) | 2 | 0 | 196 (99%) |
| Ceftazidime (n=167) | 4 | 0 | 163 (97.6%) |
| Imipenem (n=198) | 0 | 1 | 197 (99.5%) |
| Meropenem (n=190) | 0 | 2 | 188 (98.9%) |
| Mynocycline (n= 192) | 105 (54.69%) | 29 (15.4%) | 58 (30.2%) |
| Ciprofloxacine (n=192) | 12 | 1 | 179 (93.2%) |
| Netilmicine (n=192) | 34 | 2 | 158 (82.3%) |
| Gentamicine (n=182) | 8 | 3 | 171 (93.95%) |

* *S: sensitive; I: intermediate; R: resistant*

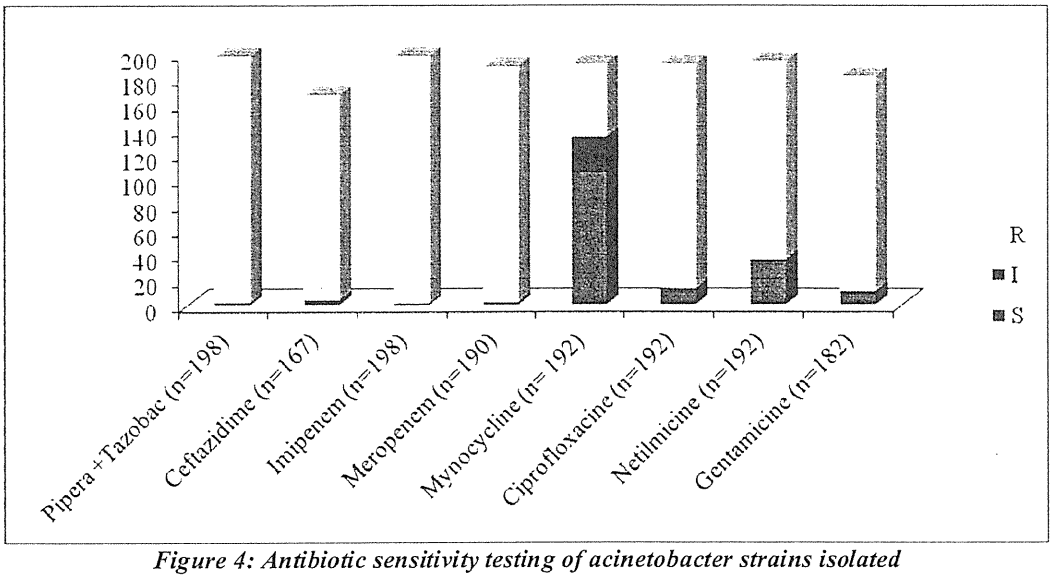


***Figure 4: Antibiotic sensitivity testing of acinetobacter strains isolated***

The results in the table 5 and Figure 4 shown, *Acinetobaer spp* isolated were resistant at high: to carbapenem level (99.5% imipenem and 98.9% to meropenem); 99% to Pipera +Tazobactam and 97.6% to ceptazidime. However 105/192 (54.69%) of strain still were sensitive to Mynocycline and 15.1% were resistant at intermediate level.

***Table 6: Antibiotic sensitivity testing of K. pneumonieae strains isolated***

| Antibiotics | Results | | | |
|---|---|---|---|---|
| | S* | I* | R* | Total |
| Amo+A.clavulanic (n=54) | 3 | 2 | 49 (90.7%) | 54 |
| Ticarcilline+A.clav (n=50) | 0 | 0 | 50 (100%) | 50 |
| Ampi+sulbactam (n=45) | 1 | 0 | 44 (97.8%) | 45 |
| Pipera +Tazobac (n=59) | 1 | 2 | 56 (94.9%) | 59 |
| Cefalothin (n=44) | 0 | 0 | 44 (100%) | 44 |
| Cefuroxime (n=54) | 1 | 1 | 52 (96.3%) | 54 |
| Cefotaxime (n=60) | 1 | 0 | 59 (98.3%) | 60 |
| Ceftazidime (n=60) | 1 | 2 | 57 (95%) | 60 |
| Imipenem (n=67) | 10 (14.9%) | 1 | 56 (83.6%) | 67 |
| Meropenem (n=58) | 7 | 0 | 51 (87.9%) | 58 |
| Ertapenem (n=46) | 1 | 0 | 45 (97.8%) | 46 |
| Ciprofloxacine (n=46) | 0 | 0 | 56 (100%) | 56 |
| Levofloxacine (n =48) | 5 | 12 | 36 (67.9%) | 53 |
| Gentamicine (n= 46) | 18 (28.1%) | 1 | 45 (70.3%) | 64 |
| Amikacine (n=44) | 16 (26.7%) | 1 | 43 (71.7%) | 60 |

* *S: sensitive; I: intermediate; R: resistant*

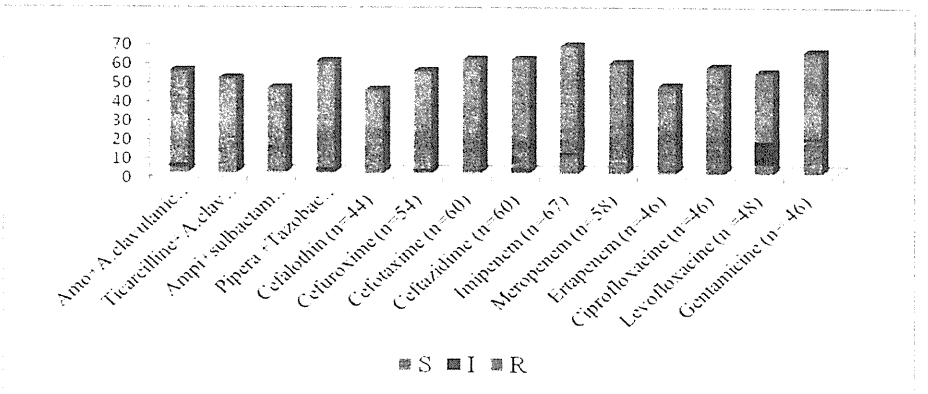


*Figure 5: Antibiotic sensitivity testing of K. pneumoniea strains isolated*

More than 96% of *K. pneumonieae* strains tested were resistant to cephalosporin and >83% to carpapenem (imipenem 83.6%, meropenem 87.9% and 97.8% to ertapenem). Other antibiotic also were reported resistant at high level (Tab 6 and Fig 5)

*Table 7 : Antibiotic sensitivity testing of E. cloacae strains isolates*

| Antibiotic | Results (n=19) | | |
|---|---|---|---|
| | S* | I* | R* |
| Amo+A.clavulanic | 0 | 0 | 19 (100%) |
| Ticarcilline+A.clav | 0 | 0 | 19 (100%) |
| Pipera +Tazobac | 0 | 1 | 18 (94.7%) |
| Cefalothin | 0 | 0 | 19 (100%) |
| Cefuroxime | 0 | 0 | 19 (100%) |
| Cefotaxime | 0 | 0 | 19 (100%) |
| Ertapenem | 1 | 1 | 17 (89.47%) |
| Imipenem | 7 (36.84%) | 3 | 9 (47.36%) |
| Meropenem | 2 | 3 | 14 (73.68%) |
| Ciprofloxacine | 2 | 2 | 15 (78.94%) |
| Gentamicine | 2 | 1 | 16 (84.2%) |

** S: sensitive; I: intermediate; R: resistant*

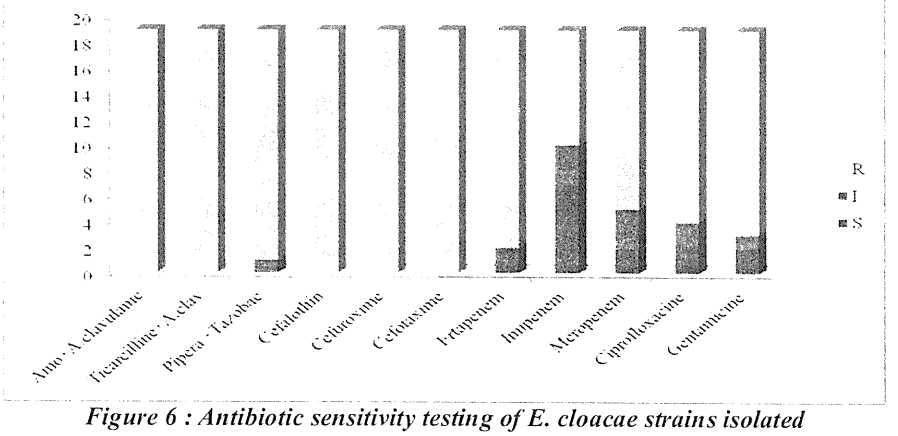


*Figure 6 : Antibiotic sensitivity testing of E. cloacae strains isolated*

100% of E. cloacae were resistant to Amo+A.clavulanic, Ticarcilline+A.clav, cephalosporin, 94.7% to Piperacilin +Tazobactam. >73% were resistant to ertapenem and meropenem. However 7/19 (36.84%) strains were still sensitive to imipenem (Tab 7 and Fig 6).

*Tables 8 : Antibiotic sensitivity testing of Pseudomonas strains isolated*

| Antibiotic | Results | | |
|---|---|---|---|
| | S* | I* | R* |
| Pipera +Tazobac (n=65) | 16 (24.6%) | 5 | 44 (67.7%) |
| Ceftazidime (n=62) | 3 | 14 | 45 (72.6%) |
| Imipenem (n=66) | 2 | 0 | 64 (96.9%) |
| Meropenem (n=66) | 5 | 0 | 61 (92.4%) |
| Ciprofloxacine (n=64) | 4 | 1 | 59 (92.2%) |
| Gentamicine (n=66) | 1 | 6 | 59 (89.4%) |
| Amikacine (n=66) | 4 | 2 | 60 (90.9%) |

* *S: sensitive; I: intermediate; R: resistant*

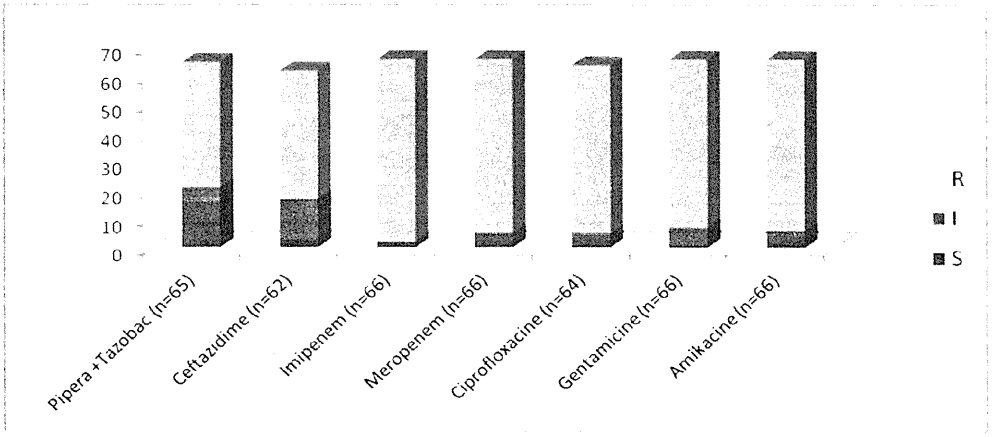


*Figure 7: Antibiotic sensitivity testing of Pseudomonas strains isolated*

*Tables 9 : Antibiotic sensitivity testing of E. coli strains isolated*

| Antibiotic | Results | | |
|---|---|---|---|
| | S* | I* | R* |
| Amo+A.clavulanic | 0 | 0 | 25 (100%) |
| Ticarcilline+A.clav | 0 | 1 | 24 |
| Pipera +Tazobac | 0 | 0 | 25 (100%) |
| Cefalothin | 0 | 0 | 25 (100%) |
| Cefuroxime | 0 | 0 | 25 (100%) |
| Cefotaxime | 0 | 0 | 25 (100%) |
| Ceftazidime | 0 | 0 | 25 (100%) |
| Ceftriazone | 0 | 0 | 25 (100%) |
| Cefepime | 0 | 0 | 25 (100%) |
| Ertapenem | 2 | 0 | 23 (92%) |
| Imipenem | 11 (44%) | 1 | 13 (52%) |
| Meropenem | 6 | 4 | 15 (60%) |
| Ciprofloxacine | 0 | 0 | 25 (100%) |
| Levofloxacine | 0 | 0 | 25 (100%) |

** S: sensitive; I: intermediate; R: resistant*

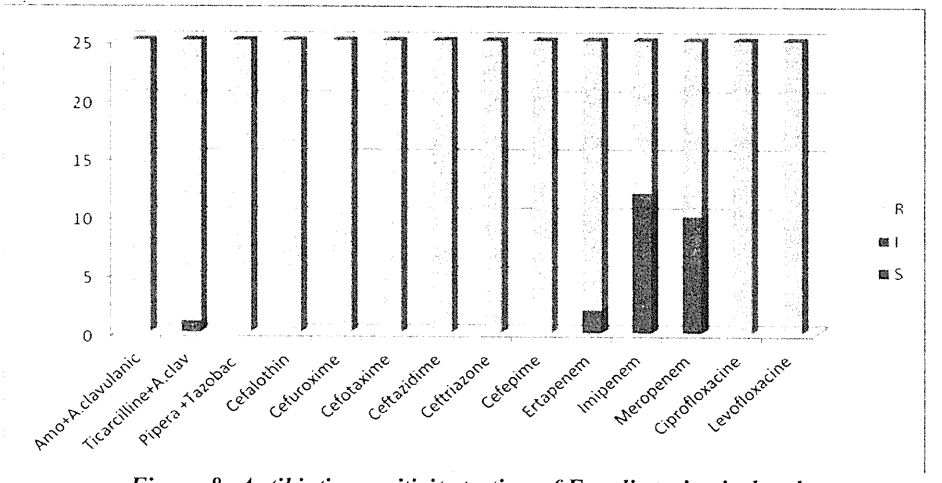


*Figure 8: Antibiotic sensitivity testing of E. coli strains isolated*

## 2.9. Discussion

- Continue finish questionnaire form, entry data and analyze the epidemiological of the study subject (from March to May, 2012)
- Compare of antibiotic resistant level among bacteria isolated in three hospitals
- Complete antibiotic sensitivity of bacteria strains isolated by Disc Diffusion method and the minimum inhibitory concentration assay (March to May, 2012)
- Evaluate the Metallo-Beta-Lactamase producing among NDM-1 positive strains isolated by E-test and Japanese kit (One Japanese Scientist from Department of Bacteriology II in NIID will visit NIHE in May, 2012 and working with Antimicrobial Laboratory-Department – NIHE).
- Discuss with NIID about new research grant for objective 2 (conduct in2012 and 2013).

## 2.10. Publications

None