Annual Report

2014

IOM-NCGM
Research Collaboration Office

March 2015
Kathmandu, Nepal
Tokyo, Japan

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Preface

The Medical Education Project, which was implemented from 1980 to 1996, contributed to the establishment and enhancement of basic and the clinical medicine of the Institute of Medicine (IOM) at Tribhuvan University along with its attached teaching hospital (TUTH). During the project period experts dispatched from NCGM worked together with IOM staff and good relationship was built between the two institutions.

Eighteen years have passed since completion of the project. During this period IOM achieved further development and currently it is greatly contributing to medical education, medical care, researches and human resource development in Nepal. Besides, reliable relationship between NCGM and IOM has been maintained until today. This is one of the valuable outcomes and legacies of the project.

In January 2013, Memorandum of Understanding (MOU) was concluded between NCGM and IOM to start a unique cooperation focusing on research and related human resource development. Two years have passed since the start of the new cooperation between NCGM and IOM. Collaborations initiated based on the MOU are on track, and a number of fruitful outcomes have been obtained. In December 2014, “the 2nd Joint Conference on Infectious Diseases with Growing Concern in Recent Years in Nepal” was held at IOM and such outcomes were presented.

It is a great pleasure for us to summarize the outline report of our new collaboration as Annual Report 2014 following the first publication of Annual Report 2013. We also would like to thank all those who worked hard for the success of the project and toward the realization of new collaborations between NCGM and IOM. I sincerely hope that the relationship of mutual trust between the two institutions will be further strengthened.

Hiroshi Ohara, M.D.,Ph.D.

Director, Division of Global Networking,
Bureau of International Health Cooperation
National Center for Global Health and Medicine
Tokyo, Japan

त्रिभुवन विश्वविद्यालय
चिकित्सा शास्त्र अध्ययन संस्थान
डीनको कार्यालय, महाराजगंज
पो.ब.नं.: १५२४, काठमाडौं, नेपाल ।
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पत्र संख्या / Ref. No.:-

Tribhuvan University
Institute of Medicine
Office of the Dean
Maharajgunj, P. O. Box: 1524
Kathmandu, Nepal
Ph.# 4410911, 4412040, 4413729, 4418187

मिति / Date.:-

Jan 27th 2015

Preface

We are delighted to know that Dr. Hiroshi Ohara, National Center for Global Health and Medicine (NCGM) is writing an annual report (2014) of our collaboration between NCGM and Tribhuvan Univrsity, Institute of Medicine. We would like to express our sincere thanks to NCGM for helping and promoting our research activites toward infection control and prevention. Over the period of time, we have had a meaningful outcomes of the collaboration in the field of research such as genotyping of Multidrug Resistant Pathogens, their characterization and situation of nosocomial infection in Nepal. Scientific conferences have been arranged in Tribhuvan Univrsity, Institute of Medicine, Kathmandu to provide platform for the scientists to share their research outcomes. In the scientific meeting, scientists from Japan and Nepal had presented their findings of research studies.

In addition, we would like to express our gratitude to all members of NCGM, specially to Dr. Hiroshi Ohara, Dr. Teruo Kirikae and other members of NCGM, who had worked hard to make it success.

Prof.Dr. Bharat Mani Pokhrel
Assistant Dean

Prof.Dr. Basista Prasad Rijal
Head
Department of Microbiology

Prof.Dr. Jeevan B. Sherchand
Director,
Research Department

Abbreviations

COPD	Chronic Obstructive Pulmonary Disease
CT	Computed Tomography
DM	Diabetes Mellitus
ESBL	Extended Stratum beta Lactamase
GFATM	Global Fund against AIDS, Tuberculosis and Malaria
IOM	Institute of Medicine
IT	Information Technology
JICA	Japan International Cooperation Agency
JOCVs	Japan Overseas Cooperation Volunteers
MBBS	Medicine Bachelor Bachelor Surgery
MOHP	Ministry of Health and Population
MOU	Memorandum of Understanding
NCD	Non Communicable Diseases
NCGM	National Center for Global Health and Medicine
NDM	New Delhi Methalo-β-Lactamase
ODA	Official Development Assistance
TUTH	Tribuban University Teaching Hospital
WHO	World Health Organization
WPRO	Western Pacific Regional Office

Contents

I. Outline of Collaboration between NCGM and IOM

1. Introduction

The Institute of Medicine (IOM) at Tribhuvan University was established as the first medical school in Nepal with the support of Japan's Grant Aid in 1980 and then a technical cooperation project (Medical Education Project) was implemented from 1980 to 1996 supported by Japan International Cooperation Agency (JICA). During this period, the National Center for Global Health and Medicine (NCGM) dispatched a project team leader, doctors and other medical professionals. Currently, IOM functions as a core in medical education, medical care and medical human resource development in Nepal, and its attached Tribhuvan University Teaching Hospital (TUTH) has gained the trust and popularity of the people in Nepal.

Recently, NCGM has begun some unique activities focused mainly on research and human resource development based on the relationship of mutual trust built between NCGM and IOM during the JICA project. At the beginning of these activities, a memorandum of understanding (MOU) was concluded between NCGM and IOM, and the IOM-NCGM Research Collaboration Office was established.

2. Before the conclusion of MOU

2-1 Grant-aid project and Technical cooperation project (1980-1996)

Since most of the medical education in Nepal was dependent on foreign countries and there were limited number of medical workers in the past, increasing the number of doctors was crucial in order to widespread modern medicine and improve the medical and hygienic standards. Hence, in 1978, the Nepalese government requested assistance from the Japanese government in order to establish a medical university.

After several surveys, a grant-aid project by JICA was initiated for the purpose of establishing a medical faculty as the 10th faculty of the Tribhuvan University located in the capital, Kathmandu. The project aimed to widely provide medical services to the general public by strengthening and developing the medical faculty as a core institution for medical education and healthcare in Nepal. The project was conducted by a combination of grant aid and technical cooperation.

Tribuvan University Institute of Medicine (IOM) in 1995

For the grant aid project, main facilities such as hospital buildings, academic building for basic science and nursing school were constructed, and essential equipment was provided.

<Grant-aid project>

- 1981 Construction of outpatient building, etc.
- 1982 Construction of patients' ward, etc.
- 1990 Construction of academic building, etc.
- 1991 Extension of patient's ward, etc.
- 1992 Repair of operation theater, etc.

The Medical Education Project was conducted as a technical cooperation project in two phases. In the first phase (1980-1989), strengthening education, clinical practice, and the research infrastructure for basic medicine and clinical medicine were mainly implemented. In the second phase (1989-1996), a technical cooperation project was implemented, focusing mainly on improving medical education to have international accreditation, enhancing the function of basic medicine, clinical medicine, and research, as well as improving hospital management function.

Technical guidance in nephrology (1996)

International Symposium on Infectious and Tropical diseases (1996)

<Technical cooperation project: Medical Education Project>

Medical Education Project Phase 1 (1980-1989)

- Dispatch of Japanese Experts 12 (14JOCVs are included)
- Invitation for Nepalese Trainees 29
- Equipment Provision

Medical Education project Phase 2 (1989-1996)

- Dispatch of Japanese Experts 86
- Invitation for Nepalese Trainees 28
- Equipment Provision

Local and International Seminar/ Symposium held by the Project

- Nepal-JICA Joint Symposium on Cholelithiasis 6-8 January 1991
- Congress on Altitude Medicine and Physiology 8 April 1992
- Nursing Research Conference 2-4 November 1993
- International Symposium on Obstructive Jaundice 17-18 February 1994
- International Symposium on Diabetes Mellitus 23-24 March 1995
- International Symposium on Infectious and Tropical Diseases 20-21 march, 1996

During the 16 year technical cooperation period almost all the basic and advanced techniques regarding diagnosis, examination, treatment and clinical record, which were indispensable to the daily medical care and medical education in IOM/TUTH, were guided (However, nosocomial infection control was not included as awareness on it was still low in those days even in advanced countries). IOM/TUTH had grown to be a medical institution that carried out a kernel function of Nepalese medical care, and had gained extremely high reliance from Nepalese people.

IOM/TUTH developed to be an internationally acknowledged medical institute; the MBBS (medicine bachelor bachelor surgery) degree awarded by IOM, Tribhuvan University was recognized by dental and medical councils of Bangladesh and Pakistan, and medical councils of India and Sri Lanka. Graduates of IOM had already pursued post-graduates education in Japan, Thailand, United Kingdom, U.S.A., and other countries.

As a result of the technical cooperation project over 16 years, the foundation for a medical education system, hospital management, clinical medicine, research, and nursing education was constructed at IOM.

2-2 After Completion of the Technical Cooperation Project (1996-2013)

The Medical Education Project was completed in July 1996. Since then, IOM has greatly contributed to healthcare in Nepal, gaining the trust of the people as the most important medical institution in Nepal. Human resources trained by the technical cooperation project play leading roles not only in the capital Kathmandu but throughout the nation, furthermore globally, greatly contributing to the medical and

healthcare field in Nepal.

After completion of the project, based on the good relationship developed during the project, NCGM and IOM have conducted small scale collaborative research activities on hepatitis, helicobacter infection, diarrhea, multi-drug resistant bacteria in respiratory tract infections, etc.

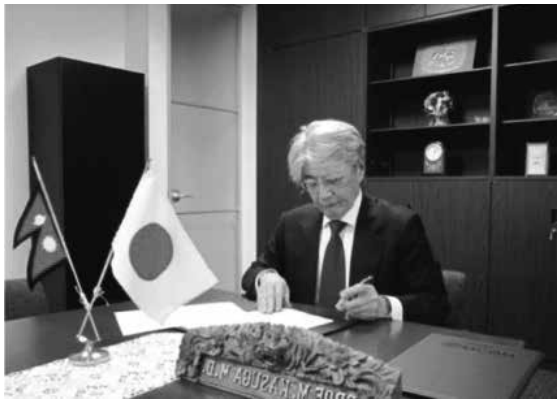
Nepal suffered political instability from 1996 to 2006 followed by a transition from the Kingdom of Nepal to the Federal Democratic Republic of Nepal in 2008. The political instability affected economic growth and compromised the delivery of social and public health interventions in the country. During this period, cooperative relations between IOM and NCGM were temporarily suspended, but with the stabilizing of political conditions in recent years, the good relationship has been recovering.

In September 2009, a joint symposium on nosocomial infection control was held at IOM jointly organized by NCGM and IOM, resulting in greater awareness of the importance of infection control and research collaboration. At the conference, successful technical cooperation in nosocomial infection control in Vietnam was introduced inviting director of Bach Mai Hospital in Hanoi. In 2013, "the 1st Joint Conference on Infectious Diseases with Growing Concern in Recent Years in Nepal" was held at IOM.

Joint Symposium on nosocomicao infection Control (September 2009)

3. Conclusion of MOU and start of new cooperation (2013-)

On January 18, 2013, NCGM concluded an agreement of cooperation with IOM in Nepal for research and related human resource development, and IOM became the fifth overseas platform for NCGM. The Memorandum of Understanding (MOU) was signed by the President of NCGM in Tokyo firstly, and then, was signed by the Dean of IOM at IOM in the capital Kathmandu on January 18, 2013. Based on this MOU, unique activities of NCGM, such as collaborative research on infectious diseases, dual burden of infectious diseases and non-communicable diseases, and development of human resources related to these researches came to be actively promoted, seeking to improve healthcare in Nepal. Collaboration between NCGM and IOM including related institutions (Kathmandu University School of Medicine, Clinics in Kathmandu, etc.) mainly on research field will be continued hereafter. Conclusion of MOU is considered to be highly effective for the smooth and efficient implementation of collaborations including related institutions.



Signing of MOU at NCGM and IOM (January 2013)

In September 2013, the IOM-NCGM Research Collaboration Office was established in the academic building of IOM, and the necessary equipment (desk, chair, computer, projector and scanner) was installed. One assistant was employed and given an orientation. Currently, activities utilizing grants from the International Health Cooperation Research (24-5, 24-5, 25-7), a grant from the Ministry of Health, Labor and Welfare of Japan, are progressing utilizing the office as a platform. IOM's Department of Microbiology and NCGM's Bureau of International Medical Cooperation manage the office operation.

The persons in charge of divisions of basic and clinical medicines are the professors for the department of Microbiology and Internal Medicine, respectively. In addition, there is a Research Department to manage research, which serves as a counterpart.

IOM-NCGM Research Collaboration Office

4. Purpose of the Collaboration between NCGM and IOM and expected outcomes

The purpose of this collaboration is to contribute to the healthcare in both countries by collaborative research and related human resource development, and to strengthen a reliable relationship.

Expected Outcomes

1. To strengthen research activities by the publication and presentation at academic conferences, etc. of the collaborative research by IOM and NCGM.
2. To obtain research results effectively through collaboration with IOM that is the main medical care and medical education institution in Nepal.

3. To provide benefits to younger staff members of NCGM by learning about the actual conditions of medical care in developing countries and how international collaborations are conducted.
4. To promote a relationship by utilizing a good relationship built on the results of the technical cooperation project.
5. To contribute to the quality improvement of medical care in Nepal by conducting researches focused on high-priority infectious diseases in Nepal and providing related technical assistance. These results are also beneficial for medical care in Japan.
6. To enable wider range of cooperation in the future, because the inclusion of other medical institutions other than IOM allows providing other possible collaboration fields.
7. To contribute to expansion of the benefit of ODA projects.

II. Current IOM

1. General information

IOM was established as the first medical school in Nepal in 1980. Thereafter the number of medical schools (faculty of medicine, medical university) increased and at the time of project termination in 1986 there were 4 medical schools in Nepal. As of January 2014, the number of medical schools in Nepal is 22, in which 7 are affiliated with IOM. Post-graduate curriculums including diploma and degree programs have been improved so as to provide better medical personnel who can contribute efficiently to medical service.

Since its inception, IOM has produced more than 1,700 medical doctors (as of 1996 it was 307). These doctors are working, not only in Kathmandu Valley, but also in rural and remote areas of Nepal, and rendering valuable services. Beside, increasing number of doctors are studying or working in foreign countries. It is noteworthy that not a few graduates of IOM are working in medical schools across the country as professors. Similarly, nursing and co-medical staff trained in IOM/TUTH are rendering medical care to the Nepalese people. IOM is carrying out quite important role in medical care as well as medical education in Nepal.

Table 1 shows comparison of hospital general information between 1985 and 2014

2. Clinical Medicine

TUTH is a general hospital which has 750 beds (as of December 2014) under 20 Clinical Departments. The equipment which was provided during the project period has been gradually replaced with new ones, but not a few old equipment are still used. Advanced equipment such as CT scan, MRI, hemodialyzers are also equipped purchased by Nepalese government. TUTH can provide advanced medical care as well as essential medical care and examinations. TUTH has been conferred duties and roles as the most important top referral medical institution in Nepal. Since 1990, Cardiovascular Center, Obstetrics & Gynecology Center and Emergency Center have been constructed by Nepalese Government.

Tribuvan University Teaching Hospital (TUTH)

The number of out-patients, in-patients, and surgical operation has been ever increasing since its establishment, which means that the importance of TUTH in Nepalese medical care has been increasing year by year.

3. Basic Medicine

Basic Medicine consists of 8 departments, which are responsible for giving lectures and practical studies to MBBS students and a variety of post-graduate courses to MBBS graduates. These departments are integrated in the academic building which was completed in 1992.

Research activities are another important role of the Basic Medicine departments in the university. The efforts to establish the basis for conducting research were made during the project period, and currently active researches are being done (currently more than 200 researches are conducted in IOM/TUTH). The results of research activities which were conducted in IOM/TUTH have been presented in various domestic and international medical congresses and journals.

The Journal of Institute of Medicine, which was founded by IOM in 1984 as the first medical journal in Nepal developed to be a high level journal carrying a lot of valuable medical papers and it has become internationally acknowledged journal. Besides the 8 basic medicine departments, Research department, Department of Information Technology and National Center for Health Professional Education were set up after the project termination.

Academic building on IOM

4. Prospects

As a result of the 16-year technical cooperation, IOM/TUTH has developed to be one of the best medical institutions which receives the deep reliance of the nation and makes many contributions to medical care in Nepal. In addition, the friendly relationship between Nepalese staff and Japanese staff, and what is more, the Nepalese nation and the Japanese nation, has been firmly established. It is quite important to make the utmost effort to maintain these good results. It is true that the nationwide expectation for IOM/TUTH as well as duties and roles has been increasing. However, IOM/TUTH has problems such as small out-patient clinic space, obsolete equipment along with increasing number of non-communicable diseases cases. Hereafter, more and more importance in the quality of medical care will be attached. We expect IOM/TUTH will make every possible effort in order to live up to these expectations, addressing the existing challenges.

Table 1 Situation and activities of IOM/TUTH : Comparison between 1985 and 2014

		1985	2014
1	Total No. of beds	401	740
2	No. of charity beds	40	63
3	No. of outpatients served (per year)	229,516	367,252
4	No. of in-patients served (per year)	11,973	22,050
5	Average length of hospitalization (days)	NA	6.73
6	No. of operations (per year)	5,237	12,228
7	Average bed occupancy rate (%)	NA	77.2
8	Number of Clinical Departments	13	20
9	Number of Basic Science Departments (including forensic medicine and public health)	8	8
10	No. of students (per school year grade)	40	75
11	Research department	-	01
12	Department of Information Technology	-	01
13	National Center for Health Professional Education	-	01

III. Research Activities

1. General information

To implement collaborative activities and strengthen the IOM-NCGM Research Collaboration Office, the following grants from the International Health Cooperation Research, grants from the Ministry of Health, Labor and Welfare of Japan were utilized in 2014.

1. 24-5: Studies on factors and trends of infectious diseases with growing concern in recent years in Nepal and Vietnam
2. 25-5: Studies on enhancement of human resource development utilizing the IOM-NCGM Research Collaboration Office - taking into consideration of the dissemination of the benefits of ODA project
3. 25-7: Fact-finding survey of nosocomial infection control in major hospitals in Nepal and planning of effective improvement

✧ The main theme of the ongoing researches is “Studies on Factors and Trends of Infectious Diseases with Growing Concern in Recent Years in Nepal.” Infectious diseases change over time, as can be clearly seen by the appearance of emerging or re-emerging infectious diseases and multi-drug resistant bacteria, and the control history of various infectious diseases by the appropriate measures. These changes in infectious diseases are considered to be associated with influential factors such as development, population movement, change in climate, nutrition, changes in the health system and disease structure, implementation of disease specific control programs, and support conditions of foreign counties. In this research, we summarized the overview of trends of infectious diseases chronologically and analyzed the factors that caused change in infectious diseases. Such overview and analysis are crucial to implement effective control measures.

As the results of preliminary study, we recognized some infectious diseases that fit this main theme. Among them we selected the following diseases and studies focusing on them were started.

(Research grant 24-5)

① Malaria control and health system
② Diarrhea caused by emerging pathogens
③ Multi-drug resistant bacteria
④ Healthcare associated opportunistic infections
⑤ Dual burden of infectious disease and non-communicable diseases

✧ Recently in Nepal, although there has been increased awareness regarding nosocomial infection control, implementation of control measures is rather slow. In the Medical Education Project, technical guidance was provided in almost all fields of medical care, but only control measures against nosocomial infection were not included in the technical cooperation subjects. This was partly because of the poor awareness for nosocomial infection control even in developed countries including Japan at that time. Nosocomial infection control is also an important research theme for NCGM-IOM collaborative research.

Effective nosocomial infection control is crucial in the healthcare facilities of developing countries, but in actual fact, attention to it is still limited and control measures are not functioning well in many countries. This study has been conducted with the purpose to investigate the actual conditions of nosocomial infection control in Kathmandu City, Nepal as a basis for the possible contribution to its improvement. (Research grants 25-7, 24-5)

✧ Collaborative researches are being conducted in Nepal utilizing the IOM-NCGM Research Collaboration Office as a base (platform). Improvement of the base functions is essential to conduct research smoothly. We are trying to strengthen the function of the IOM-NCGM Research Collaboration Office aiming at smooth implementation of researches and human resource development. 1n 2014 the following activities were conducted. (Research grant 25-5)

① Strengthening of management capacity of IOM-NCGM Collaboration Office
② Comparative study on outcomes of Medical Education Project and current IOM/TUTH
③ Preparation for enhancement of human resource development
④ Making the Annual Report 2014, NCGM-IOM Research Collaboration Office

2. Progress of research activities up to FY 2014

Research Grant: 24-5

The purpose of this study is to analyze the latest situation of the infectious diseases which have growing issues in recent years but appropriate studies along with control measures have not been done (namely, “Emerging health priorities in infectious diseases” as a new issue in the field of infectious diseases), and to discuss the cause of the spread of such diseases. This study has been carried out mainly in Nepal and partly in Vietnam with the aim of contribution to effective control of these diseases.

After the discussions with health authorities in each country, following subjects were selected and collaborative studies are going on: antibiotic resistance, health care-associated and opportunistic infections, malaria, diarrheal diseases caused by emerging pathogens (Rotavirus, Cryptosporisium, etc.), and dual burden of infectious diseases and non-communicable diseases. Necessities of conducting studies from the viewpoint of emerging health priorities for each subject are as follows:

1. Antibiotic resistance: Bacterial resistance to antibiotics is increasing however studies about the spread of multi-drug resistant bacteria in developing countries along with measures to address such situation is still limited. To provide effective guidance for control, identification of the fact and analysis of the causative factors are needed.
2. Health care-associated and opportunistic infections: These types of infections have been increasing even in developing countries alongside poor control measures, increase of compromised hosts, antibiotics abuse, application of modern technology (respirator, hemodializer, endoscope, etc.). However in general, these countries have a low awareness and control measures are not effectively done. To provide effective guidance for improvement, identification of the fact and analysis of the causative factors are needed.
3. Malaria: Progress has been made in developing countries regarding this disease under the support of the Global Fund against AIDS, Tuberculosis and Malaria (GFATM). However, in Nepal and Vietnam (also in many other endemic countries) newly developed malaria-endemic areas have been reported, and the environment and social factors have been indicated as the leading cause. It is necessary to analyze these factors in order to take effective control measures against malaria.
4. Diarrheal diseases caused by emerging pathogens: Diarrheal diseases are frequent in developing countries. In Nepal, diarrheal diseases caused by emerging pathogens such as Rotavirus, Cryptosporisium, Cyclospora are also frequently experienced at medical care settings. In order to conduct effective control, analysis of these pathogens is needed. (Basic research on the fact of these diseases and pathogens was carried out by this study group.)
5. Dual burden of communicable diseases and non-communicable diseases: These have been raised as new problems in developing countries, and their detailed analysis is needed. To address this problem it is necessary to investigate the actual conditions of the issues and identify causative factors through basic studies.

Major achievements of collaborative studies on these subjects up to now are shown below. These have been published in conference presentations, scientific papers and reports. Furthermore, through joint conferences (held in Kathmandu in January 2013, Nagasaki in October 2013, Kathmandu in December 2014) and a focal group discussions (held in Kathmandu in April 2014), study results have been shared with representatives of each country concerned (hospital personnel, WHO, Ministries of Health and Population, Embassy of Japan and JICA). Discussions have been held over the issues, and recommendations have been given based on study results.

The significance of the diseases studied have not been fully recognized, and active control measures have not been actively carried out. Our contribution to a heightened awareness by medical personnel of each country concerned can be considered as great progress. We aim to contribute to proper measures by further analyzing factors of growing issues regarding these infectious diseases (those studied by this study group).

Major achievement up to FY 2014

- Factors related to successful measures against malaria and current issues were analyzed. The results are expected to serve as useful information that contributes to measures against malaria. Besides, our in-depth study revealed that in malaria endemic-areas, the distribution rate of bed-nets was low among poverty groups which did not receive aid from the government or other countries. Also, medical facilities were not properly utilized. These are considered to be important information in order to achieve universal health coverage.
- A high frequency of diarrheal diseases caused by emerging pathogens such as Rotavirus, Cryptosporisium, and Cyclospora in Nepal was indicated.
- In medical settings in Nepal, a significant growth in drug resistance of gram negative bacilli, which cause nosocomial infections, was clearly observed. (Multiple drug resistant bacteria with strong resistance to Carbapenem and Amynoglycoside were isolated.)
- The following new findings were obtained by analyzing nosocomial pathogens:
  - The new variant of New Delhi methalo-β-lactamase producers were identified from *Escherichia coli* and named NDM-8 and NDM-12 respectively (the first case in the world).
  - "AAC (6')-Ial" gene was identified for the first time in *Serratia marcescens*,
  - "AAC (6')-Iak" gene in *Strenotrophomonas martophilia* was identified (the first case in Nepal).
  - *Providencia rettgeri* producing NDM-1 Metallo-β-Lactamase and ArmA 16S rRNA
  - Methylase was detected (the second case in the world).

- The following new findings regarding the epidemiology of extended-spectrum β-lactamase (ESBL)-producing *E. coli* were obtained: Patients infected with ESBL-producing E. coli were significantly younger than patients in Japan. Among them, infection rate was high in pregnant women. Moreover, *E. coli* 025b-ST131 clone, which is of growing global concern, was shown to have a high frequency in Nepal.
- A high rate of double burden of diabetes and tuberculosis was discovered. Furthermore, a trend toward an especially high dual burden rate of both diabetes and tuberculosis was seen among patients over the age of 40. From the above, the possibility of clinics for non-communicable diseases (NCD) and those for tuberculosis may be able to collaborate in early diagnosis of their counterpart illness.

Joint conferences and information sharing carried out in this study have contributed to increased awareness of medical personnel in each country concerned (especially in Nepal) regarding emerging health priorities of infectious diseases (multi-drug resistant bacteria, equity in the distribution of bed-nets for malaria prevention, and double burden of infectious diseases and non-communicable diseases).
Especially in Nepal, the use of antibiotics, poor countermeasures for nosocomial infections, health knowledge of local residents, environmental change caused by development processes, population movement, and lifestyle habits including diet are considered to contribute to the increase and transition of infectious diseases (studied by this research group). It is important to seek measures that address these emerging priority issues taking account of such potential factors.

This study revealed the spread of multi-drug resistant bacteria in medical settings in Nepal. Measures must be urgently taken to address this situation, in which not only medical care but also countermeasures for nosocomial infections have great influence. To implement measures effectively, active intervention not only by medical facilities but also by governmental agencies, furthermore inter sectoral collaboration, is needed.

Collaborative research on multi drug resistant bacteria in IOM

Data collection on dual burden of tuberculosis and diabetes at a clinic in Kathmandu

Research Grant: 25-5

The purpose of this study is to strengthen human resource development capacity at IOM, Tribhuvan University, by taking advantage of the overseas platform of the NCGM located at IOM (IOM-NCGM Research Collaboration Office). In the past, IOM received grant aid and technical cooperation projects from Japan's Official Development Assistance (ODA), and currently plays a leading and core role in Nepal's medical care. At IOM, studies have been carried out with the aim of sharing the benefits of medical care through human resource development, and to contribute to the improvement of medical standards in Nepal and the extension of ODA's achievements. In particular IOM focuses on improving management capacity at the IOM-NCGM Research Collaboration Office and human resource development in main areas of specialization (internal medicine and public health). In FY 2014, the following studies and activities were carried out:

1) Improvement of Management Capacity at the IOM-NCGM Research Collaboration Office:
   The IOM-NCGM Research Collaboration Office was established in January, 2013. This well-maintained office contributes to the smooth implementation of study group activities. Management of the office and liaison with other study groups have been carried out in collaboration with Department of Microbiology, Institute of Medicine, Tribhuvan University. Of particular note is the well-established framework for cooperation and the continuing good relationship with departments at the Institute of Medicine, Tribhuvan University.
   The summary of JICA's "Medical Education Project", carried out for IOM from 1980 to 1996 was prepared. Based on that summary, a comparison of achievements at hospitals and basic medical fields at the end of the project and now was carried out.

2) Improvements in Human Resource Development:
   Planning, implementation and analysis (all or in part) of the following studies were carried out with the counterpart departments, contributing to human resource development by improving study and training capacity.

- Regarding "Knowledge, Attitude and Hand washing Practices among Health Care Professionals Working in Teaching Hospital, Kathmandu, Nepal", the main study carried out by the Department of Study and Training, instructions were provided on data analysis and report preparation.
- Data analysis and report preparation of the "Study on malaria control and the equity in bed-net distribution" by the Department of Public Health were carried out with the counterpart department.

- In the “Study on pulmonary fibrosis using diagnostic imaging” carried out by Pulmonary Medicine, support was given regarding radiologic interpretation and data input, contributing to human resource development.
- Planning of “Training for Newly Employed Nurses on Measures for Nosocomial Infections” scheduled to be conducted at Nursing Department was carried out.

In December, 2014, the 2nd joint conference of three study groups (24-5, 25-5, and 25-7) using the Nepal office was held at IOM. Release of the 2014 Annual Report by March 2015 was announced.

Guidance on radiologic interpretation in TUTH

Opening address of the Dean of IOM at the 2nd Joint Conference (IOM, December 2014)

Research Grant: 25-7

Although important for the enhancement of quality in medical care, effective measures for nosocomial infection control have been limited in developing countries. The main purpose of this study group is to follow up JICA's “Training Course for Specialists of Nosocomial Infection Control and Prevention in Developing Countries” held at NCGM. This study includes Nepal, where such training has not been given up to now. In the study in Nepal, examination of actual conditions of measures for nosocomial infections, and analysis of problems was carried out, followed by important advice regarding improvement based on the obtained results and monitoring of the progress of improvement. In other words, the study in Nepal has a meaning of a negative control in contrast to the countries where training has already been provided by the above JICA’s training course.

In FY 2014, the following two surveys were carried out, followed by some advice for improvement. Further analysis of the results are planned in order to clarify the issues and points which need to be improved and to determine effective measures for improvement together with the Nepal side.

1) Examination of the Actual Condition of Measures for Nosocomial Infections in TUTH

The actual condition of nosocomial infection control were examined at the TUTH and other hospitals in Kathmandu using a questionnaire survey prepared in advance. The obtained results were compared with the results of the last survey (2012-2013).

Both the frequency of the meetings of nosocomial infection control committee and the frequency of ICT rounds by infection control teams increased. Also, some improvement in the monitoring of causative agents and in the information provision system for clinical settings were recognized. Training for newly employed nurses on nosocomial infections control was enhanced and provided to all newly hired nurses in TUTH. Issues to be addressed included improving the quality of the control system and training program, providing training to a wider range of staff members, improving waste disposal system, updating manuals, etc.

2) KAP Study on Medical Personnel regarding Nosocomial Infection Control

This study was carried out with an aim to assess the level of awareness and actual practice of “hand-washing", which is a basic prevention method of nosocomial infection control among medical personnel, and then use the obtained results for improvement. The study indicated that doctors at TUTH have a relatively good knowledge regarding hand-washing, but do not follow it in actual practice. A large number of nurses and laboratory technicians lack knowledge, and have a low degree of hand-washing in actual practice prior to contact with patients. It is important to provide improved training for medical staff regarding nosocomial infection control and prevention, which are mainly based on standard precautions.

Training course on nosocomial infection control for newly recruited nurses (TUTH)

3. Outline of each research

Table 2 shows overview of the collaborative researches between NCGM and IOM. Progress status of each research (No. 1-6) is described on pages 23-35. We have already obtained some results, and these results were published at medical conferences and on scientific papers as shown on pages 57- (papers published in 2014 were put in full paper, whereas only abstracts are put regarding papers published in 2012,-2013 and in press papers).

Among these researches it is particularly outstanding that new strain of New Delhi metalo-β-lactactamase producer was identified among the nosocomial infection cases and named NDM-8 and 12.

Discovery of NDM variants

NDM variants	Organism and country	References
NDM-1	*E. Coli* and *K. pneumoniae* (Sweden)	Yong D. et. al., 2009
NDM-2	*Acinetobacter baumanii* (Egypt)	Kaase M. et. al., 2011
NDM-3	*E. coli* (Australia)	Rogers BA. et. al., 2013
NDM-4	*E. coli* (India)	Nordmann P. et. al., 2012
NDM-5	*E. coli* (United Kingdom)	Hornsey M. et. al., 2011
NDM-6	*E. coli* (New Zealand)	Williamson DA. et. al., 2012
NDM-7	*E. coli* (Canada)	accession no. JX262694
NDM-8	*E. coli* (Nepal)	Tada T. et. al., 2013
NDM-9	*K. pneumoniae*	accession no. KC999080
NDM-10	*K. pneumoniae*	accession no. KF361506
NDM-11	Assigned (not known)	www.lahey.org/studies
NDM-12	*E. coli* (Nepal)	Tada T. et. al., 2014

Table 2 Collaborative researches in NCGM-IOM Collaboration Center, Nepal

No.	Chief Researchers in Japan and Nepal	Affiliation in Nepal	Subject	Source of fund
1	• Hiroshi Ohara (Bureau of International Medical Cooperation, NCGM) • Jeevan B Sherchand (Dept. of Public Health, Insitutie of Meidicne, Tribhuvan University)	• Institute of Medicine, Tribhuvan University • Faculty of Medicine, Kathandu University	Assessment of the interface between malaria control program and health system strengthening	24-5*
2	• Teruo Kirikae (Research Institute,NCGM) • Bharat M. Pokhrel (Dept. of Microbiology, Institute of Medicine, Tribhuvan University)	• Institute of Medicine, Tribhuvan University	Molecular epidemiology of nosocomial pathogens in developing countries	24-5*
3	• Norio Ohmagari (Disease Control and Prevention Center, NCGM) • Jatan Sherchan (School of Medicine, Kathmandu University)	• School of Medicine, Kathmandu University	Evaluation of changing epidemiology of infectious diseases in a developing country: The role of healthcare associated opportunistic infections	24-5*
4	• Shinsaku Sakurada (Bureau of International Medical Cooperation, NCGM) • Jeevan B Sherchand (Tribhuvan University, Kathmandu)	• Institute of Medicine, Tribhuvan University, • School of Medicine, Kathmandu Univeristy, • 3 clinics in Kathmandu City	Study on double burden tuberculosis (TB) and non-communicable diseases (NCD) in Nepal	24-5
5	• Hiroshi Ohara (Bureau of International Medical Cooperation, NCGM) • Bharat M. Pokhrel (Dept. of Microbiology, Institute of Medicine, Tribhuvan University, Nepal)	• Institute of Medicine, Tribhuvan University	Fact-finding survey of nosocomial infection control in major hospitals in Nepal and discussion on effective improvement plans	24-5* 25-7*
6	• Hiroshi Ohara (Bureau of International, Medical Cooperation, NCGM) • Bharat M. Pokhrel (Dept. of Microbiology, Institute of Medicine, Tribhuvan University)	• Institute of Medicine, Tribhuvan University, • School of Meicine, Kathmandu University	Studies on enhancement of human resource development utilizing the IOM-NCGM Collaboration Center - taking into consideration of the dissemination of the benefits of ODA project	25-5*

* grants from the International Health Cooperation Research, a grant from the Ministry of Health, Labor and Welfare of Japan

Research No.1

1.	Title(in English)	Assessment of the interface between malaria control program and health system strengthening
2.	Title(in Japanese)	マラリア対策とヘルスシステム強化に関する研究
3.	Main researcher	Hiroshi Ohara (Bureau of International Medical Cooperation, National Center for Global Health and Medicine)
4.	Co-Researcher(s)	Jeevan B. Sherchand (Dept. of Public Health, Institute of Medicine, Tribhuvan University, Nepal) Jatan B. Sherchan (Dept. of Microbiology, Faculty of Medicine, Kathmandu University)
5.	Resource of fund	Grants of National Center for Global Health and Medicine (24-5)
6.	Affiliation(s) in Nepal	Department of Public Health, Institute of Medicine, Tribhuvan University,
7.	Period of the research	January 2012- March 2014
8.	Publications in FY 2014	Oral presentation Successful factors contributed to malaria control and key challenges in Vietnam 29th Annual Meeting of the Japan Association for International Health, November 2014, Tokyo 1. Assessment of Health systems in relation to interface between malaria control programs and health system strengthening: comparative study among Lao PDR, Nepal and Viet Nam
9.	Summary: Malaria has been a high priority issue in many tropical and sub-tropical countries. In order to implement malaria control program effectively, it is crucial to utilize health system effectively. In this study, interactions between malaria control program and health system strengthening was assessed. The studies were conducted in Nepal and Vietnam with the methods of key informant interviews, investigation in malaria endemic areas and document review. As retrospective study, encountered challenges in malaria control and interventions for them were analyzed from the viewpoint of interactions between disease specific program and general health system using the 6 Building Blocks of Health System Strengthening of WHO (Leadership and Governance, Service delivery, Workforce, Information system, Medical products and technologies, and Financing). In addition, current challenges in malaria control were identified and possible interventions were discussed. In Nepal, malaria was showing high morbidity and mortality rate until the middle of 1990s, however thereafter it decreased remarkably due to the effective control program. Leading factors contributed to the successful control were identified as the best practices. The followings were recognized as leading current challenges in malaria control in Nepal: 1) Increase of malaria in some areas associated with population movement, 2) Shortage of health manpower in remote areas, 3) Poorly developed reporting system from the private health sector, 4) Difficulty in treatment due to increasing resistance of *P. falciparum* to anti- malaria drugs, 5) Low incentive for health workers, 6) existence of inequity of bednets distribution.	

	In Vietnam, leading good practices included: 1)Strong government commitment for malaria control, 2)National strategy for rural development and intensified education for residents, 3)Effective vertical system from national to village level for malaria surveillance and service delivery, 4)Domestic antimalarial production and high coverage of control measures, 5)Strengthening the capacity of health workers along with mobilization of mass organizations, and 6)Support from international organizations. Effective implementation under the strong leadership of the governments utilizing the existing health system was outstanding in both countries. Besides, strengthening of the vertical health program appeared to have a good impact on the general health system, particularly at the primary level. We made an in-depth study in 4 districts in Terai areas in Nepal with the aim to examine variation in utilization of bednets by socioecomomic groups and inequities in access to malaria control services. This study revealed the wider disparity and pro-rich inequities in ownership of bednets. In area without bednet intervention, ownership was significantly higher in the rich households. There was significant variation in bednet ownership across caste/ethnic groups. Disparity in ownership between the poorest and richest group appeared to be smaller in area with bednet intervention and people equally use bednets irrespective of caste and ethnic background. Free mass distribution of bednets allowed equitable ownership and reduce the inequality in usage of bednets across socioecomomic groups. The results suggested that if provided freely, bednet distribution program will be an important opportunity to reduce socioecomomic inequity in usage by allowing equitable ownership among the households of malaria risk area.

Research No.2

1.	Title(in English)	Molecular epidemiology of nosocomial pathogens in developing countries
2.	Title(in Japanese)	開発途上国の医療機関で分離される多剤耐性菌の推移に関する研究
3.	Main researcher	Teruo Kirikae (Department of Infectious Diseases, Research Institute, National Center for Global Health and Medicine)
4.	Co-Researcher(s)	Bharat M. Pokhrel (Dept. of Microbiology, Institute of Medicine, Tribhuvan University, Nepal)
5.	Resource of fund	Grants of National Center for Global Health and Medicine (24-5)
6.	Affiliation(s) in Nepal	Department of Microbiology, Institute of Medicine, Tribhuban University,
7.	Period of the research	April 2012- March 2015
8.	Publications In FY 2014	1. Antimicrob Agents Chemother. 2014, 58(10):6324-6327 2. Antimicrob Agents Chemother. 2014, 58(10):6302-6305 3. BMC Infect Dis. 2014, 14:56

9. Summary:

Emergenece of multidrug-resistant pathogens has become one of the most serious problems in medical settings worldwide. There are serious concerns about dissemination of multidrug-resistant nosocomial pathogens in Nepal.

We started a study project of drug resistant pathogens isolated from inpatients hospitalized in Tribuvan University Teaching Hospital, Kathomandu, Nepal, in collaboration between Department of Microbiology, Institute of Medicine, Tribhuvan University and National Center for Global Health and Medicine from April 2012. Professor Dr. Bharat M. Pokhrel, his colleagues and his students obtained a total of 308 Gram-negative isolates by November 2014, including *Acinetobactor baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Providencia rettgeri, Serratia marcesces* and *Stenotrophomonas maltophilia* isolates.

Of *E. coli* clinical isolates, we detected two novel NDM-type metallo-β-lactamase variant, NDM-8 and NDM-12. A novel New Delhi metallo-β-lactamase variant, NDM-8 has substitutions at positions 130 (Asp to Gly) and 154 (Met to Leu) compared with NDM-1. Expression of the *bla*NDM-8 and *bla*NDM-1 genes in *E. coli* DH5α conferred resistance or reduced susceptibility to all cephalosporins, moxalactam, and carbapenems. NDM-8 showed enzymatic activities against β-lactams similar to those of NDM-1. A novel New Delhi metallo-β-lactamase variant, NDM-12, was identified in a carbapenem-resistant *E. coli* clinical isolate obtained from a urine sample of a patient in Nepal. NDM-12 differed from NDM-1 by two amino acid substitutions (M154L and G222D). The profiles of enzymatic activities of NDM-12 against β-lactams tested were similar to those of NDM-1, although NDM-12 had lower k_{cat}/K_m ratios for all β-lactams tested except for doripenem. The lower k_{cat}/K_m ratios were likely to be caused by the lower kcat values of NDM-12 compared with those of NDM-1, as the values of NDM-12 were 11.4 to 73.6% of those of NDM-1. The bla_{NDM-12} gene was located in a plasmid of 160 kb. The sequence surrounding bla_{NDM-12} was similar to that of the pGUE-NDM plasmid from the *E. coli* strain GUE, which was isolated in India.

We reported that multidrug-resistant *Klebsiella pneumoniae* clinical isolates with Various Combinations of Carbapenemases (NDM-1 and OXA-72) and 16S rRNA Methylases (ArmA, RmtC and RmtF) were disseminating in a medical setting in Nepal. Of 25 clinical isolates *Klebsiella pneumonia* obtained in 2012, 17 were resistant to imipenem and meropenem with MICs ≥4 mg/L, and 20 were highly resistant to arbekacin, amikacin and

	gentamicin with MICs ≥512 mg/L. The carbapenemase encoding genes *bla*NDM-1 and *bla*OXA-72 were observed in 17 and 9 isolates, respectively; and the 16S rRNA methylase encoding genes *armA, rmtC* and *rmtF* were observed in 9, 5, and 9 isolates, respectively. Of *S. maltophilia* clinical isolates, we detected a novel 6'-*N*-aminoglycoside acetyltransferase encoding gene, aac(6')-Iak. The encoded protein, *AAC(6')-Iak*, consists of 153 amino acids and has 86.3% identity to AAC(6')-Iz. *Escherichia coli* transformed with a plasmid containing *aac(6')-Iak* exhibited decreased susceptibility to arbekacin, dibekacin, neomycin, netilmicin, sisomicin and tobramycin. Thin-layer chromatography showed that AAC(6')-Iak acetylated amikacin, arbekacin, dibekacin, isepamicin, kanamycin, neomycin, netilmicin, sisomicin and tobramycin. Of *S. marcescens* clinical isolates, we detected a novel 6'-N-aminoglycoside acetyltransferase-encoding gene, *aac(6')-Ial.* The encoded protein AAC(6')-Ial has 146 amino acids, with 91.8% identity to the amino acid sequence of AAC(6')-Ic in *S. marcescens* SM16 and 97.3% identity to the amino acid sequence of AAC(6')-Ian in *S. marcescens* WW4. The minimum inhibitory concentrations of aminoglycosides for *E. coli* expressing AAC(6')-Ial were similar to those for E. coli expressing AAC(6')-Ic or AAC(6')-Ian. Thin-layer chromatography showed that AAC(6')-Ial, AAC(6')-Ic, or AAC(6')-Ian acetylated all the aminoglycosides tested, except for apramycin, gentamicin, and lividomycin. Kinetic assays revealed that AAC(6')-Ial is a functional acetyltransferase against aminoglycosides. The *aac(6')-Ial* gene was located on a chromosomal DNA.

Research No.3

1.	Title(in English)	Evaluation of changing epidemiology of infectious diseases in a developing country: The role of healthcare associated opportunistic infections
2.	Title(in Japanese)	途上国における感染症の変貌と要因に関する研究 - 特に医療に関連した日和見感染に関する検討 -
3.	Main researcher	Norio Ohmagari (Disease Control and Prevention Center, National Center for Global Health and Medicine)
4.	Co-Researcher(s)	Jatan Sherchan (Department of Medical Microbiology, Kathmandu University, School of Medical Sciences, Nepal) Kayoko Hayakawa (Disease Control and Prevention Center, National Center for Global Health and Medicine) Maki Nagamatsu (Disease Control and Prevention Center, National Center for Global Health and Medicine)
5.	Resource of fund	Grants of National Center for Global Health and Medicine (24-5)
6.	Affiliation(s) in Nepal	Department of Medical Microbiology, Kathmandu University, School of Medical Sciences
7.	Period of the research	September 2012- March 2015
8.	Publications in FY 2014	Epidemiology of Extended-spectrum β-Lactamase (ESBL)-producing *Escherichia coli* in Nepal: Predominance of CTX-M-15-type ESBL. Sherchan JB, Hayakawa K, Ohmagari N, Kirikae T, Nagamatsu M, Tojo M, Miyoshi-Akiyama T, Ohara H, Sherchand JB, Tandukar S. Presented as a poster presentation at Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC, Sep, 2014. Differences in the clinical and microbiological characteristics of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* between Japan and Nepal. Hayakawa K, Sherchan JB, Kirikae T, Nagamatsu M, Tojo M, Miyoshi-Akiyama T, Ohara H, Sherchand JB, Tandukar S, Ohmagari N, Presented as a poster presentation at Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC, Sep, 2014. Clinical epidemiology and molecular analysis of Extended-spectrum β-Lactamase (ESBL)-producing *Escherichia coli* in Nepal: Characteristics of *E. coli* Sequence Type ST131 and ST648 Sherchan JB, Hayakawa K, Miyoshi-Akiyama T, Ohmagari N, Kirikae T, Nagamatsu M, Tojo M, Ohara H, Sherchand JB, Sarmila Tandukar S. Manuscript under review.
9.	Summary: The objective of this study is to evaluate the clinical epidemiology of opportunistic healthcare associated infections as well as infections due to drug-resistant pathogens in a developing country. The information would be useful to seek for the effective preventing methods for healthcare associated infections and/or infections due to drug-resistant pathogens. Information pertaining to opportunistic healthcare associated infections in developing countries are limited, and thus, this research would provide the valuable information. In addition, infections due to drug-resistant pathogens pose serious public threat worldwide. The results from Nepal where there has been sparse information on clinical epidemiology of drug resistant pathogens would be beneficial in terms of providing	

	the epidemiology of global spread of resistant pathogens and their impact. We completed the analyses of the epidemiology of drug-resistant pathogens, especially on world pandemic Extended-spectrum β-Lactamase (ESBL)-producing *Escherichia coli* in Nepal. We combined the information of clinical and microbiological characteristics of this pathogen in Nepal. The results were presented as a featured poster-walk abstract at Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC, Sep, 2014. The manuscript is currently under review. The major findings are that total of 105 patients with ESBL-*E. coli* isolation were identified, and majority (90%) of the ESBL-*E. coli* isolates were CTX-M-15 positive. The most dominant ST was ST131 (n=54 [51.4%]), followed by 15 (14.3%) cases of ST648, on which there has been few literature. Among the 54 ST131 isolates, all 54 (100%) were identified as O25b-ST131 clone. Higher proportion of ST648 isolates had resistance to non-beta lactam antibiotics than ST131 or non-ST131/648, and possessed drug resistant genes more frequently than ST131 or non-ST131/648. More than 38% of ESBL-*E. coli* were isolated from outpatient clinic, and pregnant patients consisted 24% of ESBL-*E. coli* cases. In addition, we are currently analyzing the data on the comparison of epidemiology of the resistant organisms between in Nepal and Japan. These analyses would enable to identify the key epidemiological factors associated specifically to opportunistic healthcare associated infections due to drug-resistant pathogens in a developing country.

Research No.4

1.	Title(in English)	Study on double burden tuberculosis (TB) and non-communicable diseases (NCD) in Nepal
2.	Title(in Japanese)	ネパールにおける結核と非感染性疾患の二重負荷に関する研究
3.	Main researcher	Shinsaku Sakurada (Bureau of International Medical Cooperation, NCGM)
4.	Co-Researcher(s)	Takanori Hirayama (JATA/RIT), Yuko Tsuda (Health Bureau of Osaka City), Shyam K Shrestha (FSB Clinic, Kathmandu), Jatan B Sherchan (Kathmandu University School of Medicine, Dhulikhel), Kishor S Manandhar (CF Clinic, Kathmandu), Jeevan B Sherchand (Tribhuvan University, Kathmandu)
5.	Resource of fund	Grants of National Center for Global Health and Medicine (24-5)
6.	Affiliation(s) in Nepal	Department of Public Health, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal
7.	Period of the research	September, 2012-March, 2014
8.	Publications in FY 2014	Oral Presentation: Joint Conference on Infectious Diseases with growing Concern in Recent Years in Nepal, December 2014.
9.	Summary: NCD in developing countries has increased in recent decades and may affect control of communicable diseases in particular TB. In Nepal incidence of TB has recently increased in elderly populations (>65 years old) but decreased in young populations. This trend may inevitably lead Nepali people to double burden of TB and NCD. Our objective is to survey the present status of TB and NCD in Kathmandu by epidemiological and sociological methods. We conducted a clinic-epidemiological study at three clinics in Kathmandu city. One sampling site was a clinic of respirology expert, another two sampling sites were general clinics but one of those was located in the thickly populated area with Tibetan refugees. We used use simple questionnaires with written informed consent to detect double burden cases. Then, we conducted focus group discussion (FGD) with heath officials, medical doctors, public health nurses and DOTS providers in Kathmandu. We collected 414 TB samples and 649 NCD samples from three sites. Preliminary analysis has shown high prevalence of TB among NCD patients in one general clinic located in the commercial area of Kathmandu city. While the estimated prevalence of TB in Nepal, 2010 was 238 per 100,000 populations, but that in the general clinic was 2,800 per 100,000 populations (the number of newly diagnosed patients was 8 in 282 NCD patients). Among NCDs diabetes mellitus (DM) is most common except chronic respiratory diseases. There was ten-folds higher TB prevalence among NCD patients. In FGD medical doctors agreed that the awareness of double burden is important in the both sides of patients and health care providers. They have a plan to distribute posters to clinics in Kathmandu to aware the double burden. The results of this study might contribute to measures against TB control in a new era with double burden with NCD. Since November in 2014, we have conducted a new study over the prevalence of DM in the both new and relapse cases of TB to evaluate the effect of DM in relapse of TB. We have performed OGTT for 258 new and 139 relapse cases after getting informed consent. The study will be completed by the end of March in 2015.	

Research No.5

1.	Title(in English)	Fact-finding survey of nosocomial infection control in major hospitals in Nepal and planning of effective improvement
2.	Title(in Japanese)	ネパールの主要病院における院内感染対策の実情分析と効果的な改善策に関する検討
3.	Main researcher	Hiroshi Ohara (Bureau of International Medical Cooperation, National Center for Global Health and Medicine)
4.	Co-Researcher(s)	Pokhrel BM, Shrestha RK, Dahal RK, Mishra SK, Kattel HP, Rijal BP (Dept. of Microbiology, Institute of Medicine, Tribhuvan University, Nepal) Jeevan B. Sherchand (Dept. of Public Health, Institute of Medicine, Tribhuvan University, Nepal) Shreshta DL (Dept. of Nursing Management, Tribhuvan University, Nepal) Teruo Kirikae, Yumiko Haneishi (Bureau of International Medical Cooperation, National Center for Global Health and Medicine)
5.	Resource of fund	Grants of National Center for Global Health and Medicine (24-5, 25-7)
6.	Affiliation(s) in Nepal	Department of Microbiology, Institute of Medicine, Tribhuban University,
7.	Period of the research	September 2012- March 2016
8.	Publications in FY 2014	Oral presentation 88th General Assembly of the Japanese Society of Infectious Diseases, June 2014, Fukuoka, Japan Scientific Paper *J Inst Med* 2014, 36(3): 38-48.
9.	Summary: In developing countries, where the incidence of infectious diseases is high and environmental conditions of healthcare facilities are poor, nosocomial infections may frequently occur. Effective nosocomial infection control is crucial in the healthcare facilities of developing countries, but in actual fact, attention to it is still limited and control measures are not functioning well in many countries. This study has been conducted with the purpose to investigate the actual conditions of nosocomial infection control in Kathmandu City, Nepal as a basis for the possible contribution to its improvement. 1. Fact-finding survey of nosocomial infection control in hospitals in Kathmandu, Nepal- a basis for improvement The actual condition of nosocomial infection control were examined at the TUTH and five other referral hospitals in Kathmandu City with the method of questionnaire survey and interview. The obtained results were compared with the results of the last survey (2012-2013). Both the frequency of the meetings of nosocomial infection control committee and the frequency of ICT rounds by infection control teams increased. Also, some improvement in the monitoring of causative agents and in the information provision system for clinical settings were recognized. Training for newly employed nurses on nosocomial infections control was enhanced and provided to all newly hired nurses in TUTH. Issues to be	

addressed included improving the quality of the control system and training program, providing training to a wider range of staff members, improving waste disposal system, updating manuals, etc.

These findings clearly reflect that there is a need of further improvement of nosocomial infection control program in the hospitals of capital. Moreover, nosocomial infection control program should also have aim to estimate mortality, morbidity, additional financial burden and length of stay in the hospital due to nosocomial infection for public awareness.

3) KAP Study on Medical Personnel regarding Nosocomial Infection Control

This study was carried out with the aim to assess the level of awareness and actual practice of “hand-washing", which is a basic prevention method of nosocomial infection control among medical personnel, and then use the obtained results for improvement. 163 medical personnel (doctors, nurses and laboratory technicians) at Tribhuvan University Teaching Hospital (TUTH) were subjects of this study. A questionnaire, direct observation at important departments, and discussions were used. The following results were obtained

Knowledge: 74.1% of doctors had an accurate knowledge of hand-washing (significance, timing, methods and effects), while only 19.7% of nurses and 50.0% of laboratory technicians had such knowledge.

Attitude: 83.3% of laboratory technicians, 59.3% of nurses and 29.6% of doctors considered that they had an accurate knowledge. Regarding motive of hand-washing, fear for infections during medical practices accounted for a relatively high proportion (55.5% in doctors). Reasons given for not practicing hand-washing as instructed were: “I was busy” (46.0%), “I thought it wasn’t necessary because I wore gloves” (33.1%), “Something urgent came up” (11.7%). 38.7% answered that hand-washing is important. Poor levels of hand-washing practice among newly hired personnel and the importance of education for newly hired staff members were indicated.

Practice: The following results were obtained regarding the degree of hand-washing in actual practice: “Prior to contact with patients” (55.8%), “After contact with patients” (97.5%), and “At the end of work" (96.1%). Practice levels were higher in nurses than in doctors. Drying and wiping methods were: hand dryer (52.7%), shared-use towels (22.7%), personal towel or handkerchief (12.3%), and natural drying (3.7%).

The study indicated that doctors at TUTH have a relatively good knowledge regarding hand-washing, but do not follow it in actual practice. A large number of nurses and laboratory technicians lack knowledge, and have a low degree of hand-washing in actual practice prior to contact with patients. It is important to provide improved training for medical staff regarding nosocomial infection control and prevention, which are mainly based on standard precautions.

Research No.6

1.	Title(in English)	Studies on enhancement of human resource development utilizing the IOM-NCGM Collaboration Center — taking into consideration of the dissemination of the benefits of ODA project
2.	Title(in Japanese)	ネパール拠点を活用した人材育成能力強化に関する研究 - ODA プロジェクトの成果拡大を視野に入れて
3.	Main researcher	Hiroshi Ohara (Bureau of International Medical Cooperation, National Center for Global Health and Medicine)
4.	Co-Researcher(s)	Bharat M. Pokhrel Jeevan B. Sherchand (Dept. of Public Health, Institute of Medicine, Tribhuvan University, Nepal) Karbir N. Yogi (Dept. of Pulmonology, TUTH) Mitsuhiro Kamimura (Dept. of Pulmonology, National Disaster Medical Center) Pradeep Shrestha (Dept. of Internal Medicine, TUTH)
5.	Resource of fund	Grants of National Center for Global Health and Medicine (25-5)
6.	Affiliation(s) in Nepal	Department of Public Health, Institute of Medicine, Tribhuvan University,
7.	Period of the research	April 2013- March 2015
8.	Publications in FY 2014	Annual Report of the IOM-NCGM Collaboration Office in 2014 was made.
9.	Summary: Collaborative researches are being conducted in Nepal utilizing the IOM-NCGM Collaboration Office as a base. Strengthening of the base functions is essential to conduct researches smoothly. This study was started to strengthen the function of the IOM-NCGM Collaboration Office aiming at smooth implementation of researches and human resource development. In FY 2014 the following activities were conducted. 1. Strengthening of management capacity of IOM-NCGM Collaboration Office: Management system to conduct researches/activities was clarified and some instructions were conducted to local staff. Management of the office and liaison with other study groups have been carried out. 2. Comparative study on outcomes of Medical Education Project and current IOM/TUTH: The overview of the Medical Education Project, which was implemented as Official Development Assistance by JICA from 1980 to 1996, was summarized and the situations of IOM/TUTH at the end of the project and the present time were compared. 3. Improvements in Human Resource Development: Planning, implementation and analysis (all or in part) of the following studies were carried out with the counterpart departments, contributing to human resource development by improving study and training capacity.	

	• Regarding “Knowledge, Attitude and Hand washing Practices among Health Care Professionals Working in Teaching Hospital, Kathmandu, Nepal”, the main study carried out by the Department of Study and Training, instructions were provided on data analysis and report preparation. • Data analysis and report preparation of the “Study on Malaria Control and the Equity in Bed-Net Distribution” by the Department of Public Health were carried out with the counterpart departments. • In the “Study on Pulmonary Fibrosis using Diagnostic Imaging” carried out by Pulmonary Medicine, support was given regarding radiologic interpretation and data input, contributing to human resource development. • Planning of “Training for Newly Employed Nurses on Measures for Nosocomial Infections” scheduled to be conducted at Nursing Department was carried out.

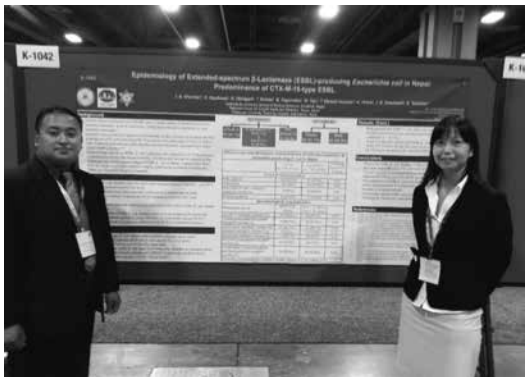
IV. Other Activities in FY 2014

1. Joint Conference

The "2nd Joint Conference on Infectious Diseases with Growing Concern in Recent Years in Nepal" was held at IOM (December 5, 2014). Following inaugural speeches by representatives of IOM and NCGM, 12 presentations on collaborative research results were made followed by questions & answers and active discussions. A total of 54 participants (Tribhuvan University counterparts, doctors and nurses from related fields, university teaching staff in Kathmandu, a professor from Australia) including four doctors from Japan were attended. The program and abstracts are shown on pages 39-53.

2. Presentation on Collaborative Research Results

A research group completed the analyses of the epidemiology of drug-resistant pathogens, especially on world pandemic Extended- spectrum β- Lactamase (ESBL) - producing *Escherichia coli* in Nepal. The results were combined with the information of clinical and microbiological characteristics of this pathogen in Nepal. These results were presented as a featured poster-walk abstract at Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC, U.S.A. in September 2014.



Regarding the study on "Assessment of the interface between malaria control program and health system strengthening", which are conducted in both Nepal and Vietnam, a counterpart researcher was invited from Vietnam in November 2014 and gave a presentation at the 29th Japan Association for International Health Congress, Tokyo. (In 2012 and 2013, a counterpart researcher in Nepal made presentations in Japan on this subject)

3. Overview of the Medical Education Project and current IOM/TUTH

The overview of the Medical Education Project, which was implemented as Official Development Assistance (ODA) by JICA from 1980 to 1996, was summarized and the situations of IOM/TUTH at the end of the project and the present time were compared.

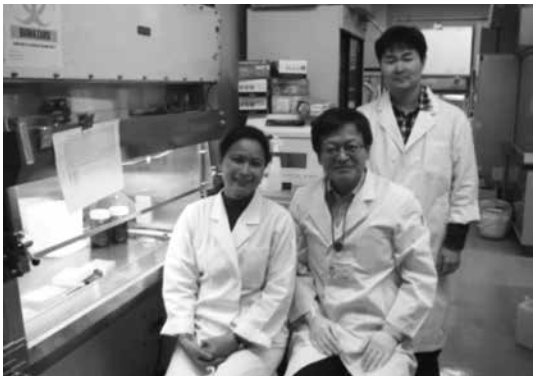
4. Enhancement of Human Resource Development

After consulting with Research Department, Department of Microbiology, Department of Public Health and Department of Internal Medicine, Department of Pulmonology the following activities were initiated, focusing mainly on improving research ability (25-5, 24-5).

1) Research Department: Hearing on the overall impact of JICA project and current problems was conducted and it found that research methods, especially, epidemiological methods and diagnostic imaging methods were weak.

2) Department of Microbiology:

Training for analysis of drug-resistant bacteria was conducted to the staff members of Department of Microbiology as a part of human resource development activities. In addition to technical instruction at IOM, NCGM invited two graduate students from the department to the Department of Infection Control at NCGM and gave technical instructions from October to November in 2014 (24-5). These 2 graduates will continue to do research based on techniques learned, with the goal of obtaining PhD degrees.



Technical guidance at NCGM to the staff of Department of Microbiology, IOM

3) Department of Internal Medicine, Department of Pulmonology:

Following provision of the necessary equipment such as computers and scanners, NCGM in collaboration with National Center for Disaster Medicine started to give assistance to conduct a research entitled “Case study of interstitial pneumonia at Tribhuvan University Teaching Hospital”. Japanese Doctor provided technical guidance on medical imaging diagnosis method (chest X-ray and chest CT) to the department staff.

Ward round in Pulmonology Dept.

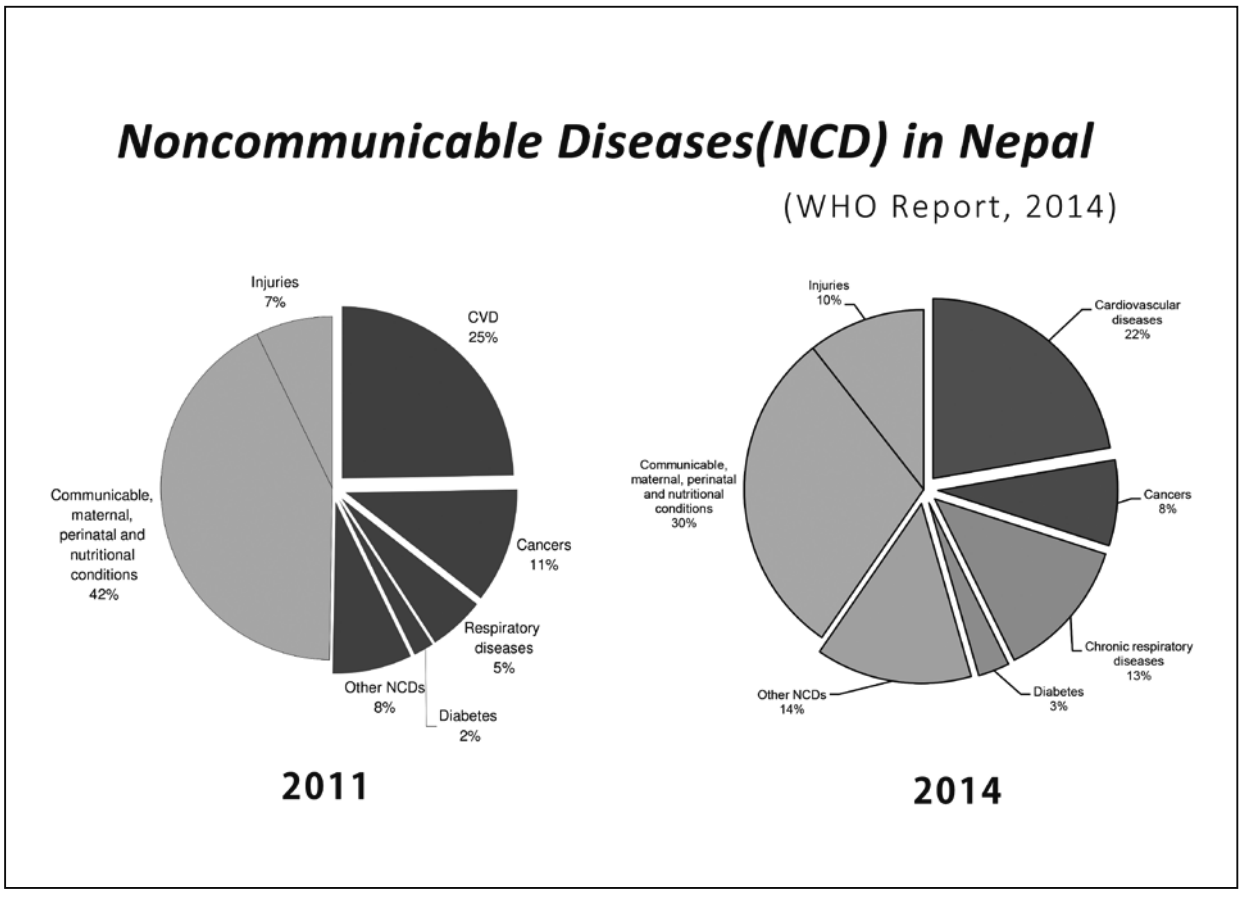
5. Overview of current medical situation in Nepal

Nepal is divided into 14 administrative zones and 75 districts and zonal and district hospitals are located by these administrative divisions. Primary healthcare centers and health posts are located under these hospitals, constructing a system that promotes medical care and hygiene among residents.

Major diseases are infectious diseases, perinatal disorders, and malnutrition. However, the frequency of non-communicable diseases such as diabetes, cardiovascular diseases, hypertension, and cancer is also increases in recent years. Common infectious diseases include diarrheal diseases, respiratory

infections, tuberculosis, hepatitis, HIV/AIDS, etc. Vector borne infectious diseases such as malaria, dengue fever, visceral leishmaniasis, and Japanese encephalitis are mainly distributed in the southern plains. Malaria has decreased remarkable during the past 15 years, however dengue fever is on the increase. In the capital Kathmandu, chronic obstructive respiratory diseases have been increasing along with the progress of air and water pollution caused by the rapid increase in population and automobiles.

Maternal mortality ratio decreased from 530 (1996) to 281 (2006) per 100,000 births, and mortality ratio of infants under five years decreased by 48% from 118 to 61 per 1,000 births between 2001 and 2005.



2nd Joint Conference on Infectious Diseases with Growing Concern in Recent Years in Nepal

Date: December 5, 2014

Venue: Institute of Medicine (IOM), Tribhuvan University, Kathmandu, Nepal

Registration: 8:00- 8:30

(Master of Ceremony: Ms. Shovita Shrestha, Dept. of Microbiology)

8:30	Introduction of participants
8:40	Inauguration by lighting the lamp: Prof. Rakesh P. Shrivastav, Dean, IOM
8:45	Remarks: Prof. Rakesh P. Shrivastav, Dean, IOM
8:50	Remarks: Prof. Jeevan K. Shrestha, Campus Chief
8:55	Remarks: Prof. Bimal K. Sinha, Asst Dean, IOM
9:00	Remarks: Prof. Bharat M. Pokhrel, Asst Dean, IOM
9:05	Remarks: Prof. Sarala Shrestha, Asst Dean, IOM
9:10	Remarks: Prof. Sharad R. Onta, Asst Dean, IOM
9:15	Remarks: Prof. Dipak P. Mahara, Executive Director, TUTH
9:20	Remarks: Dr. Teruo Kirikae, Director, Dept. of Infection Control, NCGM

Session A: ***Overview of Collaboration and Health Issues***

(Chairperson: Prof. Bharat M. Pokhrel)

9:25-9:45 ***A-1***: Overview of collaboration between IOM and NCGM

(Dr. Hiroshi Ohara, NCGM)

9:45-10:05 ***A-2***: Multidrug resistance- an emerging health issue in Nepal

(Prof. Basista Rijal IOM)

Session B : ***Multi-Drug Resistant Bacteria***

(Chairpersons: Prof. Basista Rijal, Dr. Kayoko Hayakawa)

10:20-10:35 ***B-1***: Emergence of multidrug-resistant Gram-negative nosocomial pathogens in Nepal

(Dr. Teruo Kirikae, NCGM)

10:35-10:55 ***B-2***: Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* isolates in a university hospital in Nepal

(Shovita Shrestha, IOM)

10:55-11:15 ***B-3***: Molecular epidemiology of multidrug-resistant *Escherichia coli* isolates in hospitals in Nepal

(Basudha Shrestha, IOM)

11:15-11:35 ***B-4***: Epidemiology of Extended-spectrum β-Lactamase (ESBL)-producing *Escherichia coli* in Nepal: Predominance of CTX-M-15-type ESBL

(Dr. Jatan B. Sherchan, Kathmandu Univ.)

Session C: ***Healthcare Associated Infection Control***

(Chairperson: Dr. Teruo Kirikae)

11:35-11:50 ***C-1***: Fact-finding survey of nosocomial infection control in leading hospitals in Kathmandu 2014

(Prof. Bharat M. Pokhrel, IOM)

11: 50-12:05 ***C-2***: Current situation and challenges of trainings on infection control for newly recruited nurses in TUTH

(Ns. Dharma L. Shrestha, TUTH)

12:05-13:00 Lunch

Session D: ***Diarrheal Diseases, Malaria, Double Burden, Other Important Issues***

(Chairpersons: Prof. Jeevan B. Sherchand, Dr. Hiroshi Ohara)

13:00-13:15 ***D-1***: Rotavirus gastroenteritis in children in Nepal

(Prof. Jeevan B. Sherchand, IOM)

13:15-13:30 ***D-2***: Assessment of the malaria control program in relation to general health system

(Dr. Hiroshi Ohara, NCGM)

13:30-13:50 ***D-3***: The series of studies from "DOUBLE BURDEN OF TUBERCULOSIS AND NON-COMMUNICABLE DISEASES" to "PREVELANCE OF DIABETES IN TUBERCULOSIS CASES," in Kathmandu valley, Nepal

(Dr. Takanori Hirayama, Japan Anti-Tuberculosis Association)

13:50-14:05 ***D-4***: Promotion of Research Collaboration between Nepal and Japan - Clinical Study Setting in Pulmonology

(Dr. Mitsuhiro Kamimura, National Disaster Medical Center)

14:05-14:20 Discussion

(Chairpersons: Prof. Bharat M. Pokhrel, Dr. Teruo Kirikae, Dr. Hiroshi Ohara)

14:20 Closing remarks: Prof. Rakesh P. Shrivastav, Dean, IOM

Joint Conference at IOM (December 2014)

(A-1)

Overview of Collaboration between IOM and NCGM

Hiroshi Ohara

Bureau of International Medical Cooperation
National Center for Global Health and Medicine, Tokyo, Japan

Tribhuvan University /Institute of Medicine (IOM) and National Center for Global Health and Medicine (NCGM) have a long history of cooperation starting technical cooperation project supported by JICA. In view of the successful results of the project along with the intimate and reliable relationship, IOM and NCGM agreed to enter into the Memorandum of Understanding (MOU) to promote collaboration between the two institutions focusing on researches and related activities. Currently, the following researches have been conducted based on the research grants from the Ministry of Health, Labor and Welfare, Japan.

1. 24-5: Studies on causative factors and trends of infectious diseases with growing concern in recent years in Nepal and Vietnam
2. 25-5: Studies on enhancement of human resource development utilizing the IOM-NCGM Research Collaboration Center - taking into consideration of the dissemination of the benefits of ODA project
3. 25-7: Fact-finding survey of nosocomial infection control in major hospitals in Nepal and planning of effective improvement

As the results of preliminary study, we recognized some emerging health priorities in Nepal and the following subjects were selected to conduct collaborative studies (mainly infectious diseases): Diarrhea caused by emerging pathogens, Multi-drug resistant bacteria, Healthcare associated infections, Dual burden of infectious diseases and non-communicable diseases, Malaria control in relation to health system etc.
It is a great pleasure that "the 2nd Joint Conference on Infectious Diseases with Growing Concern in Recent Years in Nepal" is held in Kathmandu on December 5, 2014. In this conference we will present the results of our collaborative studies. We are strongly convinced that our collaboration will contribute to promotion of health along with medical science in both countries.

Key words: IOM, NCGM, Collaboration, MOU, Emerging health priorities

(A-2)

Multi-drug Resistance- an Emerging Health Issue in Nepal

B P. Rijal, S.K. Mishra, H. Kattel, R.Dahal, S. Sharma, N.Shah, K Parajuli, S.Khatiwada, S.Khadka, N. parajuli, S. Dhital, S. Dahal, B .shrestha, S.S. Dhakal, J.B.Sherchand and BM Pokherel

Department of Microbiology, Tribhuvan University Teaching Hospital and Maharajganj Medical college,

Institute of Medicine (IOM), Tribhuvan University, Kathmandu

Correspondence to: Basista Rijal, email: basistarijal@gmail.com

Introduction: Antimicrobial resistance, especially Multi-drug resistance (MDR) is a global and National Health problem. MDR increases morbidity, treatment failure, cost of treatment and mortality. The present study has analyzed the trend of antimicrobial resistance of the clinical isolates with special consideration to MDR and also to appropriateness of such study.

Method: The data were collected and analyzed from many published and current ongoing studies at the IOM and in Kathmandu, mostly within the 10 years.

Results: The study revealed that increase in resistance of *Escherichia coli* over a period with different classes of drug; Cephalexin (82-100%), Ceftazidime (2-100%), Cefotaxime (15-100%), Ciprofloxacin (55-82%), Gentamycin 35-58%) and , Amikacin (0-84%).The study also showed that High level of MDR *E.coil* (49-100%) and ESBL (16-83%). Similarly, the resistance pattern of *Klebsiella Pneumoniae was* also on alarming; Ceftazidime (21-100%), gentamycin (29-8 %), Amicacin (19-81%), Meropenum (0-47%). The high proportion of of K.*pneumoniae* were MDR (67-100%), ESBL (66%) and MBL (52%). Majority of the *Acinetobacter bumennii*, a emerging pathogen were resisistant to Ceftazidime (82-100%), Gentamycin (62-100), Amikacin (54-82%) , Piperacillin (77-100%) and Meropenum (35-79%),. The high level of MDR (9.6-10%), ESBL (12%) and MBL (66%) was observed.

Moreover, the percentage of MRSA was (13-66%) also shocking. However, Vancomycin resistant *Staphylococcus aureus* was not observed. Likewise, the rising pattern of drug resistance was observed to *Streptococcus pneumoniae.* The *S.pneumoniae* were resistant to Penicillin (2-10 %), Ampicillin (2-11%) Erythromycin (0-12 %) .Furthermore, High antimicrobial resistance and MDR was also observed in other Gram negative and Gram positive bacteria including enteropathogens, Pseudomonas and other pathogens.

Conclusion: The rising pattern of antimicrobial resistance and MDR is a serious threat. Strong measures to control and prevention of drug resistance is urgently needed. To cope the problem, the resistance surveillance should be done in a regular basis by following a standard method. The result of the surveillance and its impact should be disseminated to policy makers, prescribers and to communities. A strong infection control and prevention practice should be implemented in the hospitals.

Key words: Trends, antimicrobial resistance, Nepal

(B-1)

Emergence of multidrug-resistant Gram-negative nosocomial pathogens in Nepal

Teruo Kirikae[1], Hiroshi Ohara[1] , Basista Rija[2], Jeevan B Sherchand[2] and Bharat M Pokhrel[2]

1: National Center for Global Health and Medicine, Japan

2: Department of Microbiology, Institute of Medicine, Tribhuvan University, Nepal

Since the first antibiotic, penicillin introduced in 1940s, anti-infective drugs to prevent mortality and morbidity arising from infections have been one of the most effective health interventions in the history of modern medicine. Drug-resistant pathogens were gradually emerging at first, but suddenly they were disseminating rapidly in medical settings. The current situation is becoming serious with an increasing incidence of detection of highly drug-resistant pathogens to all known drug treatments. An example is that of common bacterial infections in hospital settings. Infections with carbapenem-resistant pathogens are on the rise, and have recently become resistant to 'last-resort antibiotics'. These bacteria are an increasing cause of mortality in many countries. According to a WHO report the "…world is heading towards a post-antibiotic era in which many common infections will no longer have a cure and once again kill unabated". The selection pressure exerted by the antibiotic gets intensified with the overuse, misuse or underuse of the antimicrobial agents. Another mechanism of drug resistance is acquisition of drug resistance mediated through plasmids or mobile genetic elements. Such resistance can be horizontally transferred from one organism to another organism, including from animal infectious agents to human pathogens. This is very common with enteric bacteria such as *Escherichia coli, Klebsiella, Acinetobacter, Pseudomonas, Enterobacter* and *Enterococcus.*

Professors Pokhrel and Sherchan and their colleagues at Institute of Medicne, and Teaching Hopspital, Tribhuvan University, noticed the existence of multidrug-resistant nosocomial pathogens in hospitals in Nepal. They did surveillance of drug-resistant pathogens at first time in Nepal. Since 2012, Drs. Kirikae and Ohara at National Center for Global Health and Medicine, join their research in Nepal.

We have conducted an international collaboration study on molecular epidemiology of multi-drug resistant nosocomial pathogens. During the study period, Dr.s Kirikae and Ohara visited IOM more than ten times and Professors Pokhrel and Sherchan visited Japan twice and once, respectively. An instructor, Rajan Kumar Dahal, an M.Sc. student, Manoj Kumar Shah stayed in NCGM, 2013. Two PhD students, Basudha Shrestha and Shovita Shrestha stayed in NCGM twice 2014. We successfully achieved our goals set for our first study project. We will show a part of the results of the study in this conference.

(B-2)

Molecular epidemiology of multidrug-resistant Acinetobacter baumannii isolates in a university hospital in Nepal

Shovita Shrestha[1], Tatsuya Tada[2], Tohru Miyoshi-Akiyama[3], Kayo Shimada[2], Kazuhito Satou[4], Kuniko Teruya[4], Kazuma Nakano[4], Akino Shiroma[4], Jeevan Bdr. Sherchand[1], Basista Psd. Rijal[1], Takashi Hirano[4], Teruo Kirikae[2] and Bharat Mani Pokhrel[1]

1:Department of Microbiology, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal
2:Department of Infectious Diseases
3:Pathogenic Microbe Laboratory, Research Institute, National Center for Global Health and Medicine, Tokyo 162-8655, Japan;
4:Okinawa Institute of Advanced Science, Okinawa 904-2234, Japan

Objectives: To clarify the genetic and epidemiological properties of MDR *Acinetobacter baumannii* clinical isolates obtained from patients in Nepal and determine a complete genome sequence of a MDR *A. baumannii* isolates.

Methods: Acinetobacter spp. isolates obtained from single patients were screened for MDR *A. baumannii* with antimicrobial disk susceptibility tests. The whole genomes of the MDR isolates were sequenced by MiSeq (Illumina). The complete whole genome of an isolate, IOMTU433, were sequenced by PacBio RS II. Phylogenic trees were constructed from the SNP concatemers. MLSTs were deduced and drug resistant genes were detected.

Results: Fifty percent of *Acinetobacter spp*. isolates (122/246) were MDR *A. baumanni*, of which the majority were resistant to aminoglycosides, carbapenems, fluroquinolones but not colistin and tigecycline. These isolates harbored a 16S rRNA methylase gene, *armA*, and either $bla_{\text{NDM-1}}$, $bla_{\text{OXA-23}}$ or $bla_{\text{OXA-58}}$. 49.1% of the MDR *A. baumannii* are isolated from sputum. Most of the *A. baumannii* are resistant to most of the antibiotic tested. The multidrug-resistant *A. baumannii* harboured different β-lactamase genes and carbapenemase genes. All the 122 MDR *A. baumannii* isolates belonged to 15 different types of MLST. eBURST analysis of them together with Institut Pasteur's MLST database found that 5 of the 15 STs were grouped into 3 clonal complexes (CCs), including CC1 (ST1and ST623), CC2 (ST2) and CC149 (ST149 and ST622). The phylogenetic tree revealed 3 clades, CC1, CC2 and CC149, respectively. To our knowledge, the CC149 is a novel clonal complex of *A. baumannii*, to which MDR isolates are spreading in medical settings in Nepal.

Conclusions: This is the first report of a novel clonal complex, CC149, of *A. baumannii*, to which MDR isolates are spreading in medical settings in Nepal.

(B-3)

Molecular epidemiology of carbapenem-resistant *Escherichia coli* in Nepal

Basudha Shrestha[1], Tatsuya Tada[2], Tohru Miyoshi-Akiyama[3], Kayo Shimada[2], Jeevan Bdr. Sherchand[1], Basista Psd. Rijal[1], Teruo Kirikae[2*] and Bharat Mani Pokhrel[1]

1: Department of Microbiology, Institute of Medicine, Tribhuvan University, Nepal

2: National Center for Global Health and Medicine, Japan

The emergence of carbapenem-resistant Enterobacteriaceae (CRE) isolates producing class B metallo-β-lactamases (MBLs) has become a major concern in medical settings worldwide. MBLs are produced by many species of Gram-negative bacteria. MBLs confer resistance or reduced susceptibility to almost all β-lactams including carbapenems. New Delhi metallo-β-lactamase (NDM) -1 producing *Escherichia coli* and *Klebsiella pneumoniae* isolates was initially obtained from an Indian patient in 2008 in Sweden. Since then, NDM-1-producing *Enterobacteriaceae* has been reported in 29 countries until December 2012. Till date, 12 NDM variants producers have been found in several countries; including NDM-8 and NDM-12 producing *E. coli* from Nepal.

The purpose of this study is to determine phenotypic and genotypic properties of carbapenem-resistant *E. coli* isolates obtained from hospitalized patients in Nepal. *E. coli* isolates were screened with antimicrobial disk susceptibility tests and MICs of carbapenems were determined with the microdilution method. Whole genome sequences of carbapenem-resistant *E. coli* isolates were determined by next generation sequencers. Of 250 *E. coli* isolates tested, 39 (16%) were resistant to carbapenems. Of the 39 CRE isolates, 32 produced various NDM variants, including NDM-1 (10 isolates), NDM-3 (2), NDM-4 (1), NDM-5 (11), NDM-7 (7) and NDM-12 (1). Of the 32 NDM producing carbapenem-resistant *E. coli* isolates, 17 also produced 16S rRNA methylases, including, ArmA (8), RmtB(7) and RmtC (2), which confer high resistance against all aminoglycosides.

These results strongly suggest that carbapenem-resistant *E. coli* isolates producing both NDM-type metallo-β-lactamases and 16S rRNA methylases disseminates in medical settings in Nepal. In this study, we discovered a novel NDM variant, NDM-12 in a clinical isolate in Nepal.

Key words: Carbapenem-resistant Enterobacteriaceae (CRE), New Delhi Metallo-β-lactamase (NDM), 16S rRNA methylase

(B-4)

Epidemiology of Extended-spectrum β-Lactamase (ESBL)-producing *Escherichia coli* in Nepal: Predominance of CTX-M-15-type ESBL

J. B. Sherchan[1], K. Hayakawa[2], N. Ohmagari[2], T. Kirikae[2], M. Nagamatsu[2], M. Tojo[2], T. Miyoshi-Akiyama[2], H. Ohara[2], J. B. Sherchand[3], S. Tandukar[3]

1:Kathmandu University School of Medical Sciences, Dhulikhel. Nepal

2:National Center for Global Health and Medicine, Tokyo, Japan

3:Tribhuvan University Teaching Hospital, Kathmandu, Nepal

Background: Extended-spectrum β-lactamase (ESBL)-producing bacteria are a major threat to antimicrobial-resistant organisms worldwide. In particular, the spread of CTX-M-type ESBL-producing *Escherichia coli* (ESBLEC) is a significant public health concern. Epidemiology reports on ESBLEC in Asia are limited. Therefore, we examined the molecular and clinical epidemiology of ESBLEC in large university hospitals in Nepal.

Methods: Unique cases with isolation of ESBLEC from 2/2013 –7/2013 were included. ESBLEC isolates were screened for *pabB* and CTX-M β-lactamase genes by using O25b-ST131 clone allele-specific PCR. *E. coli* phylogenetic groups were determined by PCR.

Results: Totally, 107 ESBLEC strains were isolated during the study period. All ESBLEC isolates were collected from urine. The mean age of patients with ESBLEC was 40.8 ± 23.2 years, and 66 (61.7%) were female. In female patients, ESBLEC were more frequently identified at outpatient clinics than male patients (female patients with ESBLEC isolation at outpatient clinics: n=33 [50%] vs male patients: n=7 [17.1%]) ($p<0.001$). Male patients with ESBLEC often had underlying urological conditions such as benign prostate hypertrophy (n = 7 [17.1%]) and urolithiasis (n = 3 [7%]). Fifteen (22.7%) female patients with ESBLEC were pregnant. Of the 107 isolates, 96 (89.7%) were positive for CTX-M-15 and 55 (51.4%) were identified as *E. coli* O25b-ST131 clones. Clinical and microbiological characteristics are presented in Table 1.

Conclusion: Most of the ESBLEC isolates in Nepal were CTX-M-15 positive, and more than half were of *E. coli* O25b-ST131. The clinical and microbiological characteristics did not differ between the ST131 and non-ST131 groups. The resistance of ESBLEC to multiple classes of antibiotics as well as its prevalence in communities, especially among pregnant females, is of great concern.

(C-1)

Fact-finding survey of nosocomial infection control in leading hospitals in Kathmandu 2014

Bharat M. Pokhrel[1], Hiroshi Ohara[2], Teruo Kirikae[2], Shyam K. Mishra[1],Hari P. Kattel[3], Sangita Sharma[1], Dharma L. Shrestha[3], Yumiko Haneishi[2], Keshav P. Parajuli[1], Basista P. Rijal[1], Jeevan B. Sherchand[1]

1:Department of Microbiology,Institute of Medicine, Tribhuvan University, Nepal

2: Bureau of International Medical Cooperation, National Center for Global Health and Medicine, Japan

3:Tribhuvan University Teaching Hospital, Institute of Medicine, Nepal

Nosocomial infection is worldwide problem both in developed and developing countries. In view to assess the actual scenario of health care associated infection, 5 referral hospitals of capital of Nepal were surveyed at one time in the year 2014. Survey methods included standard questionnaire, site visits and interviews.

Briefly, questionnaire format consisted method of infection control, training situation, personal protection equipment (PPE) etc.

These hospitals had nurses ranging from 70-530 and doctors 20-250 as per bed capacity. The bed capacity of these hospital ranged 100-460. Two hospitals had infectious diseases department. Four hospitals had protocal for hospital associated infection control. Two hospitals had WHO/ CDC guide line for infection control. Three hospitals had infection control committee. One hospital holds meeting bimonthly or as per demand. Two hospitals did not have N95 mask. Whereas others responded poor availability of N95 mask. Regarding personal protective equipments; gloves, gowns and masks were sufficiently available where as goggles were poorly available. Respondents of all hospital were expecting infection control training, although in some hospitals they had in-house training.

These findings clearly reflect that there is a need of further improvement of nosocomial infection control program in the hospitals of capital. Moreover, nosocomial infection control program should also have aim to estimate mortality, morbidity, additional financial burden and length of stay in the hospital due to nosocomial infection for public awareness.

Key words: Fact-findings survey, nosocomial infection control, Leading hospitals, Kathmandu,2014

(C-2)

Current situation and challenges of trainings on infection control for newly recruited nurses in TUTH

Dharma Laxmi Shrestha
Nursing Department, TUTH

Tribhuvan University Teaching Hospital (TUTH) is a tertiary care hospital in Kathmandu. It provides specialty care in an academic setting to patients who are referred from around Nepal. TUTH provides outpatient and inpatient care and patients tend to have complex problems requiring consultation from medical and surgical subspecialties as well as intermediate and intensive levels of care (for example, ICU).

Infection control is significant challenge at TUTH for nursing staff as nursing staff provide a critical role in implementation of infection control practice. In reality, there are many challenges to effective infection control practice which results in inadequate implementation. Proper hand hygiene is not effectively practiced. Patients with transmissible infectious diseases are not able to be properly isolated. Both of these simple infection control practices contribute to nosocomial infections. Infection control training of nursing staff is performed. However, frequent turn over of nursing staff means that training has to be frequently repeated which is not always feasible or implemented. Even when nursing staff are educated regarding proper infection control practice, insufficient infrastructure for infection control is a problem. Only high care areas (ICU and MICU) have alcohol hand wash is possible the other wards, soap and water is available. There may only be one sink in an entire ward to wash hands, making it inconvenient and difficult for nursing staff to perform hand hygiene during clinical practice. Drying of hands is often used by a towel which may serve as a reservoir for microorganisms and contaminate health care worker's hands right after hand washing.

At present, there is no regular monitoring of infection control practice in the hospital. Without proper monitoring, it is difficult to understand how well we perform infection control activities .Recently hospital has going to establish a health care waste management system as recommended by WHO protocol.

(D-1)

Rotavirus gastroenteritis in children: Hospital associated molecular epidemiology in Nepal

Jeevan B. Sherchand[1] and Hiroshi Ohara[2]

1: Tribhuvan University Teaching Hospital, Institute of Medicine, Department of Microbiology and Public Health Research Laboratory, Kathmandu, Nepal

2: National Center for Global Health and Medicine, Tokyo, Japan

ABSTRACT

Rotavirus is the major pathogens of community acquired acute gastroenteritis in children, but their role in hospital acquired gastroenteritis is not fully understood. The aim of the study was to assess the incidence of hospital associated gastroenteritis and molecular evidence among hospitalized children less than 5 years of age.

Stool samples were collected from 1721 hospital admitted children under 5 years of age attending Children's Hospital with acute watery diarrhea during 2010 and 2011 and findings will be presented. But the presentation will be highlighted from suspected children with hospital acquired acute gastroenteritis. Acute gastroenteritis was classified as hospital acquired infection if diarrhea developed in 48 hours or more after admission. The incidence of hospital acquired infection due to rotavirus was 31.2% (48/ 154) by ELISA. The distribution of rotavirus genotypes G and P, serotype G12 represented 48% of rotavirus strains characterized by reverse transcription-polymerase chain reaction genotyping during the study, and was associated with P-types P[6], P[8] and P[4]. Further, a total of nine G/P type combination were identified, with G12 P [6] 30% being the most commonly detected rotavirus strain type. Most of the children who had hospital acquired rotavirus gastroenteritis found symptoms of diarrhea, vomiting, fever, poor sucking and dehydration. Additional findings showed that 2% cases of rotavirus co-infection with bacterial pathogens of *Esch coli* and *Shigella* species. The study revealed that G 12 and G12P [6] were found major genotypes causing hospital acquired rotavirus gastroenteritis in Nepal. Introduction of rotavirus vaccine along with strengthening hygienic measures could substantially reduce the incidence of hospital acquired acute gastroenteritis in children of Nepal.

Key word: Rotavirus, Gastroenteritis, Hospital associated, Nepal

(D-2)

Assessment of the malaria control program in relation to general health system

Hiroshi Ohara[1], Jeevan B. Sherchand[2], Jatan B. Sherchan[3], Takanori Hirayama[1]

1: Bureau of International Medical Cooperation, National Center for Global Health and Medicine

2: Public Health Laboratory, Institute of Medicine, Tribhuvan University

3: Department of Clinical Microbiology, Kathmandu University

Malaria control has been a major health issue with high priority in endemic countries. In this study, successful factors in malaria control and key challenges were analyzed, particularly in relation to health system.

Studies were conducted in Nepal and Vietnam. Information obtained from the document reviews, interviews, and field surveys were analyzed from the viewpoint of interface between malaria control program (vertical health system) and the general health system with special emphasis on good practices and key challenges.

Leading good practices include: 1)Strong government commitment for malaria control (Particularly in Vietnam), 2)National strategy for rural development and intensified education for residents, 3)Effective vertical system from national to village level for malaria surveillance and service delivery, 5)Strengthening the capacity of health workers along with mobilization of mass organizations, and 6)Support from international organizations.

Key challenges include: 1)Large number of population at risk in remote and mountainous areas, 2) Uncontrolled seasonal migrants and cross border movement, 3)Lack of human resources for malaria network, and 4)Increasing drug resistance of malaria parasite, 5)Inequality in distribution of bed nets (Particularly in Nepal).

Strengthening of the vertical health program appeared to have some impact on the general health system, particularly at the primary level. More efforts are requested for strengthening of the health system in remote areas, training of health staff at peripheral level, accurate quality assurance, promotion of public-private relationship and equity in bed net distribution. These tackling will directly lead to further strengthening of the general health system, and eventually effective implementation of various health programs and contribution to UHC.

Key words: Malaria, Nepal, Vietnam, Challenges, Equity

(D-3)

The series of studies from "DOUBLE BURDEN OF TUBERCULOSIS AND NON-COMMUNICABLE DISEASES" to "PREVELANCE OF DIABETES IN TUBERCULOSIS CASES," in Kathmandu valley, Nepal

Takanori Hirayama[1], Jatan B Sherchand[2], Shayam Shrestha[3], Kishor Manadhar[4], Yoko Tsuda[5], Keshav Parajuli[6], Jeevan B Sherchand[6], Sharan Gopali[7], Shinsaku Sakurada[8]

1: Japan Anti-Tuberculosis Association

2: Kathmandu Medical University

3: Friends of Shanta Bhawan Clinic

4: Chhatrapati Free Clinic

5: Osaka city Public Health Office 6: Institution of Medicine, Tribhuvan University

7: Japan-Nepal Health & TB Research Association

8: National Center for Global Health and Medicine

Non-communicable diseases (NCD) patients have increased and tuberculosis (TB) patients have increased in elderly populations in recent decades in Nepal. This trend may inevitably lead Nepali people to double burden of TB and NCD. To understand the situation, interviews about NCD situations based on the questionnaire format had been conducted for TB patients and NCD patients who have history of TB treatment at clinics. A single disease of NCD could not be probed epidemiologically as a risk of TB in this study. However, the double burden of TB and NCD was observed at high ratio. And the TB patients with NCD were older than TB patients without NCD. TB screening for NCD patients at clinics might be worth considering as an opportunity for early diagnosis.

Focus Group Discussion was conducted in January 2014. Participants were 4 Doctors, 1 Government officer, 1WHO TB Expert, and 6 Public Health Nurses. The main theme was the present situation of TB and NCDs double burden in Nepal and its associated problems. Their conclusions :1) The problem of double burden is yet unrecognized at the national level. 2) DM might be a screening target for TB patients and diabetics might be a target group for TB screening.

To study the association between DM and TB, the study about prevalence of diabetes in new and relapse tuberculosis cases had been started from November 2014.

(D-4)

Promotion of Research Collaboration between Nepal and Japan --- Clinical Study Setting in Pulmonology

Mitsuhiro Kamimura[1], Hiroshi Ohara[2]

1: Department of Pulmonology, National Hospital Organization Disaster Medical Center, 2: Bureau of International Medical Cooperation, NCGM

Key words: Pulmonology, JICA Project, Kathmandu, Nepal

The institute of medicine (IOM) of Tribhuvan University Teaching Hospital (TUTH) was supported by JICA project from 1980 to 1996, during that period IOM accepted many experts from National Center for Global Health and Medicine (NCGM). Even after the termination of JICA Project, joint research studies have been carried out by several researchers of IOM and NCGM, mainly focused on infectious diseases, gastrointestinal diseases, etc. In January 2013, MOU was concluded to expand the activities of joint researches and human resource development between IOM and NCGM. Based on this MOU, the project of "The research for strengthening of human resource development at IOM in Nepal TUTH" was started. The Pulnomology Department of National Hospital Organization Disaster Medical Center (DMC), which has strong relationship with NCGM, also has its history of mutual communication with TUTH focusing on pulmonary rehabilitation, study on bacterial contamination of endoscopies, etc. This time DMC started the joint study with Pulmonology Department of TUTH under the observation of NCGM to establish and to expand the development of better co-operation for the conduction of joint clinical studies between TUTH and DMC, NCGM and extract the factors which should be improved for better communication. The joint study is focused on the current status of interstitial pneumonitis (IP) in Nepal. As the infectious diseases have been overcome gradually in developing countries, non-communicable diseases, such as hypertension, diabetes mellitus or COPD are now emerging as new health problems. Though the prevalence is much less, IP must be a big health issue in developing countries and exact reality of the disease is not yet understood. This might be the first report in Nepal and also the factors to be solved for better performance of joint study between TUTH and Japan will be clarified through this study. Thus, we believe that this study might contribute the purpose of the project which includes activation of research communications and human resource development between Nepal and Japan.

Publications of scientific papers

Table 3 shows the list of list of papers published on international journals as Achievement in NCGM-IOM Collaboration Office

Papers published on international medical journals are shown on pages 55- (papers published in 2014 were put in full paper, whereas only abstracts are put regarding papers published in 2012,-2013 and in press papers)

Nurses in TUTH

NCGM researchers with the Dean and
Assistant Deans of IOM, Tribhuvan University

Entrance of the IOM-NCGM Research Collaboration Office
in Academic Building of IOM,

Table 3 List of papers published on international journals (papers in press are included) 2012-2014: Achievement in NCGM-IOM Collaboration Office

◎ Full papers are put in this annual report ○ Only abstract is put in this annual report

	Papers	
1	Sherchan JB, Ohara H, Sakurada S, Basnet A, Tandukar S, Sherchand JB, Bam DS. Enteric opportunistic parasitic infections among HIV-seropositive patients in Kathmandu, Nepal. Kathmandu Univ Med J 2012; 38(2):14-17.	○
2	Sherchan JB, Ohara H, Sherchand JB, Tandukar S, Sakurada S, Gurung B, Ansari S, Rijal BP, Pokhrel BM. Moleculare evidence based hospital acquired rotavirus gastroenteritie in Nepal. Prime J Mirobobiol Res 2012; 1(2): 16-21.	○
3	Shrestha S, Chaudhari R, Karmacharya S, Kattel HP, Mishra SK, Dahal RK, Bam N, Banjade N, Rijal BP, Sherchand JB, Ohara H, Koirala J, Pokhrel BM. Prevalence of nosocomial lower respiratory tract infections caused by multi drug resistant pathogens. J Inst Med 2012; 33(2): 7-14.	○
4	Tada T, Miyoshi-Akiyama T, Dahal RK, Sah MK, Ohara H, Shimada K, Kirikae T, Pokhrel BM. NDM-8 metallo-β-lactamase in a multidrug-resistant Escherichia coli strain isolated in Nepal. Antimicrob Agents Chemother 2013; 57(5): 2394-2396.	○
5	Shrestha RK, Dahal RK, Mishra SK, Parajuli K, Rijal BP, Sherchand JB, Kirikae T, Ohara H, Pokhrel BM. Ventilator associaed pneumonia in tertiary care hospital, Maharajgunj, Kathmandu, Nepal. J Inst Med 2013; 35(3): 21-28.	○
6	Ohara H, PokhrelBM, DahalRK, Mishra SK, Kattel HP, Shrestha DL, Haneishi Y, Sherchand JB. Fact-finding survey of nosocomial infection control in hospitals in Kathmandu, Nepal and trial to improvement. Tropical Med Health 2013; 41:113-119.	○
7	Tada T, Miyoshi-Akiyama T, Dahal RK, Sah MK, Ohara H, Shimada K, Kirikae T, Pokhrel BM. NDM-1 metalloβ-lactamase and ArmA 16S rRNA methylase producing Providencia rettgeri clinical isolates in Nepal. BMC Infect Dis 2014; 14:56-60	◎
8	Tada T, Miyoshi-Akiyama T, Dahal RK, Mishra SK, Ohara H, Shimada K, Kirikae T, Pokhrel BM. Dissemination of multidrug-resistant Klebsiella pneumoniae clinical isolates with various combinations of carbapenemases (NDM-1 and OXA-72) and 16S rRNA methylases (ArmA, RmtC and RmtF) in Nepal. Int J Antimicrob Agents 2014; 42(4):372-374.	◎
9	Tada T, Shrestha B, Miyoshi-Akiyama T, Shimada K, Ohara H, Kirikae T, Pokhrel BM. NDM-12, a Novel New Delhi Metallo-β-Lactamase Variant from a Carbapenem-Resistant Escherichia coli Clinical Isolate in Nepal. Antimicrob Agents Chemother 2014; 58(10):6302-6305.	◎
10	Tada T, Miyoshi-Akiyama T, Dahal RK, Shyam MK, Shimada K, Ohara H, Kirikae T, Pokhrel BM. Identification of a Novel 6'-N-Aminoglycoside Aetyltransferase, AAC(6')-Iak from a Multidrug-resistant Clinical Isolates of Stenotrophomonas maltophilia. Antimicrob Agents Chemother 2014; 58(10):6324-6327.	◎
11	Sah MK, Mishra SK, Ohara H, Kirikae T, Sherchand JB, Rijal BP, Pokhrel BM. Nosocomial bacterial infection and Antimicrobial resistant Pattern in a tertiary Care hospital in Nepal. J Inst Med 2014; 36(3): 38-48.	◎
12	Sherchan JB, Hayakawa K, MD, Miyoshi-Akiyama T, Ohmagari N, Kirikae T, Nagamatsu M, Tojo M, Ohara H, Sherchand JB, Tandukar S. Clinical epidemiology and molecular analysis of Extended-spectrum β-Lactamase (ESBL)-producing Escherichia coli in Nepal: Characteristics of E. coli Sequence Type ST131 and ST648. Antimicrob Agents and Chemother (in press)	○
13	Ohara H, Matsumoto M, Hirayama T, Akashi H, Sherchand JV, Nam VH, Sherchan JB. Assessment of health systems in relation to interface between malaria control programs and health system strengthening: Comparative study between Nepal and Viet Nam. Tropical Med Health (in press)	○

KATHMANDU UNIVERSITY MEDICAL JOURNAL

Enteric Opportunistic Parasitic Infections Among HIV-Seropositive Patients in Kathmandu, Nepal

Sherchan JB,[1] Ohara H,[2] Sakurada S,[2] Basnet A,[3] Tandukar S,[3] Sherchand JB,[3] Bam D S[4]

[1]Kathmandu University School of Medical Sciences, Dhulikhel, Nepal

[2]National Center for Global Health and Medicine, Tokyo, Japan

[3]Tribhuvan University Teaching Hospital, Public Health Research Laboratory, Kathmandu, Nepal

[4]Dirgh-Jeevan Health Care Research Center, Tripureswar, Kathmandu, Nepal

Corresponding Author

Jatan B. Sherchan

Department of Microbiology

Kathmandu University School of Medical Sciences

Dhulikhel, Nepal

Email: jatansherchan@gmail.com

Citation

Sherchan JB, Ohara H, Sakurada S, Basnet A3, Tandukar S, Sherchand JB, et al. Enteric Opportunistic Parasitic Infections Among HIV-seropositive Patients in Kathmandu, Nepal. *Kathmandu Univ Med J* 2012;38(2):14-17.

ABSTRACT

Background

Enteric opportunistic parasitic infections are the major source of diarrheal disease in developing countries mainly in Human Immunodeficiency virus (HIV) infected patients.

Objective

The study was to detect enteric parasites causing diarrhea and their association with immune status in HIV-seropositive patients.

Methods

The present study was conducted in Dirgh-Jeevan Health Care Research Center and Tribhuvan University Teaching Hospital, Public Health Research Laboratory, Kathmandu, Nepal between June 2010 and May 2011 involving 146 Human Immunodeficiency virus (HIV) positive patients. Serostatus from these patients were detected by Enzyme Linked Immunosorbent assay. CD4+ T cell counts were done by flow cytometry. Stool was examined for enteric parasites by microscopy with special staining methods.

Results

A total of 146 HIV sero-positive patients with and without diarrhea age between 20 to 45 years were included in the study. Of the 146 patients, the protozoan parasitic infection was found in 30.13% (44/146). Out of 146 patients, 78 had diarrhea in which parasitic infection was 39 (50%) and 7.35% (5/68) protozoal parasites positive cases did not have diarrhea. A significant difference ($p<0.05$) was observed in the level of infection of intestinal protozoan between the HIV seropositive with diarrhea and HIV-seropositive without diarrhea. Out of 43 patients whose CD4+ T cells were <200/µl, 29 (67.4%) had opportunistic parasitic infection whereas out of 103 patients whose CD4+ T cells were ≥200/µl, only 15 (14.56%) had opportunistic parasitic infection ($P < 0.05$).

Conclusion

Enteric opportunistic parasitic infections were detected in 30.1% among HIV-seropositive patients and low CD4+ T count indicated high enteric opportunistic infection. Early detection of enteric parasitic infections will help in the management and to improve the quality of life for HIV-infected individuals.

KEYWORDS

Diarrhea, HIV, Opportunistic parasites

INTRODUCTION

Enteric opportunistic parasitic infections are major source of diarrheal disease in developing countries mainly in HIV infected patients. The progressive decline and ultimate destruction of immune system functions, which are characteristic for AIDS, usually result in morbidity and ultimately death due to opportunistic bacterial, viral, fungi and parasitic infections.[1] Gastrointestinal infections are very common in patients with HIV infection or AIDS.[2] Diarrhea is a common clinical presentation of these infections. Reports indicate that diarrhea occurs in 30-60 % of AIDS patients in developed countries and in about 90 per cent of AIDS patients in developing countries.[3] The presence of

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Full Length Research

Hospital acquired Rotavirus Gastroenteritis and molecular evidence in Nepal

Sherchand JB[1], Ohara H[2], Sherchan JB[3], Tandukar S[1], Sakurada S[2], Gurung B[4], Ansari S[1], Rijal BP[1], and Pokhrel BM[1]

[1]Tribhuvan University Teaching Hospital, Institute of Medicine, Department of Microbiology and Public Health Research Laboratory, Kathmandu, Nepal
[2]National Center for Global Health and Medicine, Tokyo, Japan
[3]Department of Medical Microbiology, Kathmandu University School of Medical Sciences, Dhulikhel.
[4]Kanti Children's Hospital, Kathmandu, Nepal;

Accepted 4th October, 2011

Rotavirus is the major pathogens of community acquired acute gastroenteritis in children, but their role in hospital acquired gastroenteritis is not fully understood. The aim of the study was to assess the incidence of hospital acquired gastroenteritis and molecular evidence among hospitalized children less than 5 years of age. A total of 154 children with hospital acquired acute gastroenteritis in children's hospital of Kathmandu were enrolled between January and December 2010. Acute gastroenteritis was classified as hospital acquired infection if diarrhea developed in 48 hours or more after admission. The incidence of hospital acquired infection due to rotavirus was 31.2% (48/ 154) by ELISA. The distribution of rotavirus genotypes G and P, serotype G12 represented 48% of rotavirus strains characterized by reverse transcription-polymerase chain reaction genotyping during the study, and was associated with P-types P[6], P[8] and P[4]. Further, a total of nine G/P type combination were identified, with G12 P [6] 30% being the most commonly detected rotavirus strain type. Most of the children who had hospital acquired rotavirus gastroenteritis found symptoms of diarrhea, vomiting, fever, poor sucking and dehydration. Additional findings showed that 2% cases of rotavirus co-infection with bacterial pathogens of *Esch coli* and *Shigella* species. The study revealed that G 12 and G12P [6] were found major genotypes causing hospital acquired rotavirus gastroenteritis in Nepal. Introduction of rotavirus vaccine along with strengthening hygienic measures could substantially reduce the incidence of hospital acquired acute gastroenteritis in children of Nepal.

Key word: Rotavirus, gastroenteritis, hospital acquired, Nepal

INTRODUCTION

Rotavirus is the major pathogen of community acquired acute gastroenteritis in children, but their role in hospital acquired gastroenteritis and molecular evidence is not fully understood. Although rotavirus is the most frequent cause of gastroenteritis in children under 5 years of age, but, the virus can cause severe diarrhea and dehydration, especially in children aged 6 to 24 months. In developing countries, acute gastroenteritis due to rotavirus infection causes the death of approximately 440,000 children every year (Parashar *et al.* 2009; Festini *et al.* 2010 and Parashar *et al.* 2006).The rotavirus genus of the *Reoviridae* family is very diverse, as it consists of different groups (A-G) and of different types based on the characteristics of the surface proteins VP7 (G = glycoprotein) and VP4 (P = protease-sensitive protein). To date, at least 23 G types and 31 P types of group A rotavirus, the group which most commonly infects humans, have been differentiated (Festini *et al.* 2010; Kang *et al.* 2006 and Ursu *et al.* 2009).

The virus is mainly transmitted by feco-oral route or by direct contact, but it can occasionally be transmitted through droplets. Since the virus is stable in the environment, transmission can occur through the ingestion of contaminated water and food, and through

*Corresponding author. E-mail: jeevansherchand@hotmail.com

Original Article

Prevalence of nosocomial lower respiratory tract infections caused by multi drug resistance pathologens

Shrestha S, Chaudhari R, Karmacharya S, Kattel HP, Mishra SK Dahal RK, Bam N, Banjade N, Rijal BP, Sherchand JB, Ohara H, Koirala J, Pokhrel BM

Department of Microbiology, Department of Internal Medicine, Tribhuvan University Teaching Hospital, Bureau of international Cooperation, International Medical Centre of Japan, Southern Illinois University School of Medicine, USA

Corresponding author: Prof. Dr. Bharatmani Pokharel, Department of Microbiology, Tribhuvan University Teaching Hospital, Nepal

E. mail- bmp268@hotmail.com and/or shovitadhakal@live.com

Abstract

Introduction: Nosocomial infections caused by multi-drug resistant pathogens are major threat to the hospitalized patients. Extended spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) producing bacterial strains causing hospital acquired lower respiratory tract infection are increasing in numbers. Only a limited number of studies related to MBL producers have been done in Nepal.

Objective: The goal of this study was to determine the etiology of nosocomial lower respiratory tract infections and to assess the current levels of antimicrobial resistance with special reference to ESBL and MBL producing bacterial strains.

Methods: A total of 100 specimens including sputum and endotracheal secretion from patients diagnosed of nosocomial lower respiratory tract infection were collected and processed according to the standard methodology. Combination disk method was done for the detection of ESBL and MBL producing isolates.

Results: Out of total 100 specimens, 87% was monomicrobial while the rest were polymicrobial. 96.5 % were gram negative while 3.5% were gram positive. All *E.coli, Klebsiella* spp and *S. aureus* were found to be MDR followed by *Acinetobacter* spp (97.2%) and *P. aeruginosa* (76.2%)

About 28.6 % of *E. coli*, 8.33% of *Klebsiella* spp and 2.4 % of *Pseudomonas aeruginosa* were ESBL producers. *Acinetobacter* spp. was not found to produce ESBL during the study. MBL was present in 17.4% of the gram negative isolates.

Conclusion: We found a high prevalence of MDR strains as a cause of nosocomial LRTI including significant proportions of ESBL and MBL producers. The rate of *Acinetobacter spp.*, including MBL producers, in our hospital setting was alarmingly high which prompts a special attention for the management of such patients as well as urgent need for implementation of infection control strategies.

Key words: MDR, LRTI, ESBL, MBL, nosocomial infection

Introduction

Nosocomial respiratory tract infections are major cause of excessive morbidity and mortality. Patients with serious underlying diseases have an especially high risk of acquiring these infections and that risk is magnified by exposure to respiratory therapy. Until recently, contaminated respiratory care devices were a major cause of infection, but procedures

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NDM-8 Metallo-β-Lactamase in a Multidrug-Resistant *Escherichia coli* Strain Isolated in Nepal

Tatsuya Tada,[a] Tohru Miyoshi-Akiyama,[a] Rajan K. Dahal,[c] Manoj K. Sah,[c] Hiroshi Ohara,[b] Teruo Kirikae,[a] Bharat M. Pokhrel[c]

Department of Infectious Diseases, Research Institute,[a] and Department of International Medical Cooperation,[b] National Center for Global Health and Medicine, Toyama, Shinjuku, Tokyo, Japan; Department of Microbiology, Institute of Medicine, Tribhuvan University, Maharajgunj, Kathmandu, Nepal[c]

A novel metallo-β-lactamase, NDM-8, was identified in a multidrug-resistant *Escherichia coli* isolate, IOMTU11 (NCGM37), obtained from the respiratory tract of a patient in Nepal. The amino acid sequence of NDM-8 has substitutions at positions 130 (Asp to Gly) and 154 (Met to Leu) compared with NDM-1. NDM-8 showed enzymatic activities against β-lactams similar to those of NDM-1.

Metallo-β-lactamases (MBLs) produced by Gram-negative bacteria confer resistance to all β-lactams except monobactams (1). New Delhi metallo-β-lactamase-1 (NDM-1), a recently discovered MBL, was initially isolated from *Klebsiella pneumoniae* and *Escherichia coli* in 2008 in Sweden (2). Since then, NDM-1-producing members of the *Enterobacteriaceae* have been isolated in various parts of the world, including Australia, Bangladesh, Belgium, Canada, France, India, Japan, Kenya, the Netherlands, New Zealand, Pakistan, Singapore, Taiwan, and the United States (3, 4). In addition, isolates producing six NDM variants have been reported, including NDM-2-producing *Acinetobacter baumannii* strains from Egypt (5, 6), Israel (5), Germany (7), and the United Arab Emirates (8); an NDM-3-producing *E. coli* strain from Australia (accession no. JQ734687); an NDM-4-producing *E. coli* strain from India (9); an NDM-5-producing *E. coli* strain from the United Kingdom (10); an NDM-6-producing *E. coli* strain from New Zealand (11); and an NDM-7-producing *E. coli* strain from Canada (accession no. JX262694).

E. coli IOMTU11 (NCGM37) and *Pseudomonas aeruginosa* IOMTU9 (NCGM1841) were isolated from pus from a surgical site and from sputum of patients, respectively, in 2012 at Tribhuvan University Teaching Hospital in Kathmandu, Nepal. The isolates were phenotypically identified, and species identification was confirmed by 16S rRNA sequencing (12). MICs were determined using the microdilution method recommended by the Clinical and Laboratory Standards Institute (13). *E. coli* IOMTU11 was resistant to all antibiotics tested excepted fosfomycin (MIC, 4 μg/ml). The MICs of β-lactams are shown in Table 1, and those of other antibiotics were as follows: arbekacin, >1,024 μg/ml; amikacin, >1,024 μg/ml; colistin, 0.25 μg/ml; gentamicin, >1,024 μg/ml; and tigecycline, 0.5 μg/ml. MBL production was examined with an MBL Etest (Sysmex; bioMérieux Co., Marcy l'Etoile, France), with MICs of 256 μg/ml of imipenem and 2 μg/ml of imipenem-EDTA. PCR analysis for MBL genes (14, 15, 16) and 16S rRNA methylase genes (17) was performed. The isolates were positive for bla_{NDM} and *rmtB*. Sequence analysis showed that the *bla*NDM was a novel variant, and it was designated bla_{NDM-8}. Multilocus sequence typing (MLST) of IOMTU11 showed that it was ST101 (*Escherichia coli* MLST database [http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi.html]). *P. aeruginosa* IOMTU9 had bla_{NDM-1}, which was used as a reference gene.

The sequence of the bla_{NDM-8} gene showed mutations corresponding to two amino acid substitutions compared with bla_{NDM-1} (accession number JF798502). Analysis of the predicted amino acid sequence revealed two substitutions (D130G and M154L) compared with NDM-1, one substitution (D130G) compared with NDM-4, and one substitution (L88V) compared with NDM-5.

The bla_{NDM-8} and bla_{NDM-1} genes were cloned into the corresponding sites of pHSG398 (TaKaRa Bio, Shiga, Japan) with the primer set EcoRI-NDM-F (5′-GGGAATTCATGGAATTGCCCAATATTATG-3′) and PstI-NDM-R (5′-AACTGCAGTCAGCGCAGCTTGTCGGCCAT-3′). *E. coli* DH5α was transformed with pHSG398-NDM-8 or pHSG398-NDM-1 to determine the MICs of β-lactams.

The open reading frames of NDM-1 and NDM-8 without signal peptide regions were cloned into the expression vector pQE2 (Qiagen, Tokyo, Japan) with the primer set SacI-NDM-F (5′-CCCCTCGAGCAGCAAATGGAAACTGGCGACCAACGGT-3′) and SalI-NDM-R (5′-CCCGAGCTCTCAGCGCAGCTTGTCGGCCATGCGGGCC-3′). The plasmids were transformed into *E. coli* BL21-CodonPlus (DE3)-RIP (Agilent Technologies, Santa Clara, CA). The recombinant NDM proteins were purified using nickel-nitrilotriacetic acid (Ni-NTA) agarose according to the manufacturer's instruction (Qiagen). His tags were removed by digestion with DAPase (Qiagen), and untagged proteins were purified by an additional passage over Ni-NTA agarose. The purities of NDM-1 and NDM-8 were over 90%, as estimated by SDS-PAGE. During the purification procedure, the presence of β-lactamase activity was monitored with nitrocefin (Oxoid Ltd., Basingstoke, United Kingdom). Initial hydrolysis rates were determined in 50 mM phosphate buffer (pH 7.0) at 25°C with a UV-visible spectrophotometer (V-530; Jasco, Tokyo, Japan). The K_m and k_{cat} values and the k_{cat}/K_m ratio were determined by analyzing β-lactam hydrolysis by use of the Lineweaver-Burk plot. Wavelengths and extinc-

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Address correspondence to Teruo Kirikae, tkirikae@ri.ncgm.go.jp.

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Original Article

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Ventilator Associated Pneumonia in Tertiary Care Hospital, Maharajgunj, Kathmandu, Nepal

Shrestha RK*, Dahal RK*, Mishra SK*,Parajuli K*, Rijal BP*, Sherchand JB* , Kirikae T, Ohara H, Pokhrel BM***

*Department of Microbiology, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal; **National Centre for Global Health and Medicine (NCGM) Tokyo,Japan

Abstract

Introduction: Ventilator Associated Pneumonia (VAP) is the most common nosocomial infection among intensive care unit (ICU) patients and lack of much information in Nepal. So, the aim of this study was to determine prevalence and bacteriological profile of VAP with special reference to multi-drug resistant (MDR), Methicillin-resistant Staphylococcus aureus(MRSA),Metallo-β-Lactamase(MBL), Extended-Spectrum β-Lactamase(ESBL)-producing bacterial strains.

Methods: A total 150 tracheal specimens were studied during June 2011 to May 2012 at Department of Microbiology, TUTH as described by American Society for Microbiology (ASM). Combination disk method was done for the detection of ESBL and MBL producing isolates.

Results: PrevalenceofVAPwasfoundtobe34%.Acinetobactercalcoaceticusbaumanniicomplex (44%) was the commonest isolate, followed by Klebsiellapneumoniae (22%), Pseudomonas aeruginosa (16%) and Staphylococcus aureus (12%). Among MDR Gram negative bacteria (GNB), 39% were MBL and 33% were ESBL-producers. All GNB (61) were sensitive to Polymyxin B and Colistinsulphate, whereas, 48% were found resistant to Carbapenems. Prevalence of MRSA was 75%, which were all sensitive to Vancomycin.

Conclusion: High prevalence of VAP, MDR along with MRSA or ESBL or MBL producing strains was found in the study. Thus, suitable control measures must be adopted to cope up this alarming situation with genetic characterization.

Key words: VAP, ICU, MDR, MRSA, ESBL, MBL.

Introduction

People with life-threatening injuries and illnesses need critical care and mechanical ventilation is must. It is often a life-saving intervention, but carries many potential complications, including pneumothorax, airway injury, alveolar damage, collapsed lung and ventilator-associated pneumonia.[1]

Ventilator-associated pneumonia (VAP) is defined as an episode of pneumonia in a patient who requires a device to assist or control respiration through a tracheostomy or endotracheal tube at the time of or within 48 hours before the onset of the infection.[2] Eighty-six percent of nosocomial pneumoni as are associated with mechanical ventilation.[3] This is associated with increases in morbidity and mortality, hospital length of stay, and costs.

In modern medical practice, extensive use of antibiotics have resulted in emergence and rapid dissemination of Multi drug resistant (MDR), Methicillin Resistant Staphylococcus aureus (MRSA), Extended-Spectrum β-Lactamase (ESBL) and Metallo-β-Lactamase (MBL) producing bacteria. Thus, their detection is crucial for the optimal treatment of patients and to control the spread of resistance.So this study is intended to address the issues regarding the prevalence of VAP, MDR, ESBL-, MBL-producing bacterial isolates, and MRSA.

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Original article

Fact-finding Survey of Nosocomial Infection Control in Hospitals in Kathmandu, Nepal—A Basis for Improvement

Hiroshi Ohara[1]*, Bharat M. Pokhrel[2], Rajan K. Dahal[2], Shyam K. Mishra[2], Hari P. Kattel[2], Dharma L. Shrestha[3], Yumiko Haneishi[1] and Jeevan B. Sherchand[2]

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Abstract: The purpose of this study was to investigate the actual conditions of nosocomial infection control in Kathmandu City, Nepal as a basis for the possible contribution to its improvement. The survey was conducted at 17 hospitals and the methods included a questionnaire, site visits and interviews. Nine hospitals had manuals on nosocomial infection control, and seven had an infection control committee (ICC). The number of hospitals that met the required amount of personal protective equipment preparation was as follows: gowns (13), gloves (13), surgical masks (12). Six hospitals had carried out in-service training over the past one year, but seven hospitals responded that no staff had been trained. Eight hospitals were conducting surveillance based on the results of bacteriological testing. The major problems included inadequate management of ICC, insufficient training opportunities for hospital staff, and lack of essential equipment. Moreover, increasing bacterial resistance to antibiotics was recognized as a growing issue. In comparison with the results conducted in 2003 targeting five governmental hospitals, a steady improvement was observed, but further improvements are needed in terms of the provision of high quality medical care. Particularly, dissemination of appropriate manuals, enhancement of basic techniques, and strengthening of the infection control system should be given priority.
Key words: Fact finding survey, nosocomial infection control, Kathmandu, Nepal

INTRODUCTION

Recently, nosocomial infections have become a global concern recognized as a major patient safety issue. They not only cause a significant burden on patients but also lower the quality of medical care. In addition, prolonged hospitalization due to nosocomial infections increases costs and unnecessary expenses for the hospital [1, 2]. In the healthcare setting, particularly in developed countries, various measures including the organization of infection control teams (ICTs), preparation of manuals, strengthening of surveillance systems, and training of staff have been taken to assure effective control. However, it is only some decades ago that importance was attached to nosocomial infection control and effective measures were employed, even in developed countries [3].

In developing countries, where the incidence of infectious diseases is high and environmental conditions of healthcare facilities are poor, nosocomial infections may frequently occur, and some studies have reported a high incidence at healthcare facilities in these countries [4–6]. Effective nosocomial infection control is crucial in the healthcare facilities of developing countries, but in actual fact, attention to it is still limited and control measures are not functioning well in many facilities. Furthermore, as implementation of control measures seems to be costly and to consume resources, nosocomial infection control is often given a low priority.

Severe acute respiratory syndrome (SARS), which originated in Guangdong Province, China in November 2002, spread to more than 30 countries. In many hospitals where SARS cases were encountered, nosocomial infections also broke out, causing many casualties along with economic havoc [7, 8]. It is not overstatement to say that such outbreaks have heightened awareness regarding nosocomial infection control even in developing countries. In

[1] Bureau of International Medical Cooperation, National Center for Global Health and Medicine, Japan
[2] Department of Microbiology, Institute of Medicine, Tribhuvan University, Nepal
[3] Tribhuvan University Teaching Hospital, Institute of Medicine, Nepal
*Corresponding author:
Bureau of International Cooperation, International Medical Center of Japan, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162-8655, Japan
Tel: +81-3-3202-7181
Fax: +81-3-3205-7860
E-mail: oharah@it.ncgm.go.jp

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NDM-1 Metallo-β-Lactamase and ArmA 16S rRNA methylase producing *Providencia rettgeri* clinical isolates in Nepal

Tatsuya Tada[1], Tohru Miyoshi-Akiyama[1], Rajan K Dahal[3], Manoj K Sah[3], Hiroshi Ohara[2], Kayo Shimada[1], Teruo Kirikae[1*] and Bharat M Pokhrel[3]

Abstract

Background: Drug-resistant *Providencia rettgeri* producing metallo-β-lactamase and 16S rRNA methylase has been reported in several countries. We analyzed *P. rettgeri* clinical isolates with resistance to carbapenems and aminoglycosides in a hospital in Nepal.

Methods: Five clinical isolates of multidrug-resistant *P. rettgeri* were obtained in a hospital in Nepal. Antimicrobial susceptibilities were determined using the microdilution method and entire genomes were sequenced to determine drug-resistant genes. Epidemiological analysis was performed by pulsed-field gel electrophoresis.

Results: Four of the 5 isolates were resistant to carbapenems (imipenem and meropenem), with MICs ≥16 mg/L, with the remaining isolate showing intermediate resistance to imipenem, with an MIC of 2 mg/L and susceptibility to meropenem with an MIC ≤1 mg/L. All 5 isolates had bla_{VEB-1}. Of the 4 carbapenem-resistant strains, 3 had bla_{NDM-1} and 1 had bla_{OXA-72}. All isolates were highly resistant to aminoglycosides (MICs ≥1,024 mg/L) and harbored *armA*. As the result of pulsed-field gel electrophoresis pattern analysis in the 5 *P. rettgeri* isolates, 4 had identical PFGE patterns and the fifth showed 95.7% similarity.

Conclusions: This is the first report describing multidrug-resistant *P. rettgeri* strains harboring bla_{NDM-1} or bla_{OXA-72} and *armA* isolated from patients in Nepal.

Keywords: NDM-1, OXA-72, 16S rRNA methylase, *Providencia rettgeri*, Molecular epidemiology

Background

Providencia rettgeri has been associated with hospital acquired infections, including catheter-related urinary tract infections, bacteremia, skin infections, diarrhea, and gastroenteritis [1,2]. To date, there have been 5 reports of *P. rettgeri* isolates harboring metallo-β-lactamase (MBL) encoding genes, including IMP-type MBL producers in Japan [3,4]; VIM-type MBL, PER-1 extended-spectrum β-lactamase (ESBL) and 16S rRNA methylase ArmA in Korea [5]; and NDM-type MBL in Israel [6] and Brazil [7].

NDM-type MBL was initially identified in *Klebsiella pneumoniae* and *Escherichia coli* in 2009 in Sweden [8]. Since then, NDM-1-producing *Enterobacteriaceae* have been isolated in various parts of the world [9,10].

Exogenously acquired 16S rRNA methylase genes responsible for very high levels of resistance to various aminoglycosides are widely distributed among *Enterobacteriaceae* and glucose-nonfermentative microbes [11]. Gram-negative pathogens producing 16S rRNA methylase ArmA have been isolated in various countries [11].

Although co-production of several resistance determinants is not rare in *Enterobacteriaceae* [12-16], it is less common in *P. rettgeri* [5]. We describe here *P. rettgeri* clinical isolates from Nepal that produce carbapenemase (NDM-1 or OXA-72) and 16S rRNA methylase (ArmA).

* Correspondence: tkirikae@ri.ncgm.go.jp
[1]Department of Infectious Diseases, Research Institute, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku, Tokyo 162-8655, Japan
Full list of author information is available at the end of the article

Table 1 Summary of the characteristics of the 5 *P. rettegeri* strains, including antimicrobial resistance profiles and resistant genes

Strains	Tissue sources	Infection	MIC (mg/L)														Antibiotics resistant genes
			PIP	TZP	CAZ	FEP	IPM	DPM	MEM	ATM	ABK	AMK	GEN	CIP	CST	FOF	TIG	
IOMTU1	Pus	SSI	1,024	512	>1,024	64	32	16	64	1,024	>1,024	>1,024	>1,024	128	>128	512	4	bla_{NDM-1}, bla_{OXA-10}, bla_{VEB-1}, bla_{TEM-1}, bla_{ADC-67}, *armA*, *aadA1*, *aadA2*
IOMTU4	Sputum	NLRTI	1,024	128	>1,024	256	16	16	32	1,024	>1,024	>1,024	>1,024	>256	>128	512	4	bla_{OXA-72}, bla_{OXA-10}, bla_{VEB-1}, bla_{TEM-1}, bla_{ADC-67}, *armA*, *aadA1*
IOMTU91	Sputum	NLRTI	>1,024	1,024	>1,024	1,024	64	32	64	1,024	>1,024	>1,024	>1,024	256	128	128	4	bla_{NDM-1}, bla_{OXA-10}, bla_{VEB-1}, bla_{TEM-1}, bla_{ADC-67}, *armA*, *aadA1*
IOMTU94	Pus	SSI	1,024	4	>1,024	256	2	1	1	>1,024	1,024	1,024	>1,024	256	>128	1,024	4	bla_{OXA-10}, bla_{VEB-1}, bla_{TEM-1}, bla_{ADC-67}, *armA*, *aadA1*
IOMTU99	Sputum	NLRTI	>1,024	512	>1,024	128	64	32	64	1,024	>1,024	>1,024	>1,024	>256	>128	1,024	4	bla_{NDM-1}, bla_{VEB-1}, bla_{OXA-10}, bla_{TEM-1}, bla_{ADC-67}, *armA*, *aadA1*

SSI, surgical site infection; NLRTI, nosocomial lower respiratory tract infection PIP, piperacillin; TZP, piperacillin/tazobactam; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; DPM, doripenem; MEM, meropenem; ATM, aztreonam; ABK, arbekacin; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; CST, colistin; FOF, fosfomycin; TIG, tigecycline.

Methods

Bacterial strains

Five *P. rettgeri* clinical isolates were obtained from May to July 2012 from 5 patients at Tribhuvan University Teaching Hospital in Kathmandu, Nepal. Three isolates were from sputum and 2 from pus at surgical sites. Samples were obtained as part of standard patient care. Phenotypical identification [17] was confirmed by API 32GN (BioMérieux, Mercy l'Etoile, France) and 16S rRNA sequencing (1,497 bp) [18,19].

Antimicrobial susceptibilities

MICs were determined using the microdilution method, according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) [20]. Breakpoints to antibiotics were determined. The modified Hodge test, the meropenem-sodium mercaptoacetic acid double-disk synergy test (Eiken Chemical, Tokyo, Japan) and E-test (imipenem/EDTA) (AB Biodisk, Solna, Sweden) were performed.

Entire genome sequencing

The entire genomes of these isolates were extracted and sequenced by MiSeq (Illumina, San Diego, CA). CLC genomics workbench version 5.5 (CLC bio, Tokyo, Japan) was used for de novo assembly of reads and to search for 923 drug-resistance genes, including genes encoding β-lactamases, 16S rRNA methylases and aminoglycoside-acethyl/adenylyltransferases; point mutations in the *gyrA*, *parC* and *pmrCAB* operons; and point mutations in the *fos* genes, including *fosA*, *fosA2*, *fosA3*, *fosC* and *fosC2*.

Pulsed-field gel electrophoresis (PFGE) and southern hybridization

PFGE analysis was performed as described [3]. An 813 bp probe for $bla_{\text{NDM-1}}$ was synthesized by PCR amplification using the primers 5′-atggaattgcccaatattatg-cac-3′ (forward) and 5′-tcagcgcagcttgtcggccatgcggg-3′ (reverse), and a 780 bp probe for $bla_{\text{OXA-72}}$ was synthesized using the primers 5′-agtttctctcagtgcatgttcatctat-3′ (forward) and 5′-agaaccagacattccttctttcatttc-3′ (reverse). Southern hybridization to detect $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-72}}$ was performed using these probes, which were detected using DIG High Prime DNA labeling and detection starter kit II (Roche Diagnostics, Mannheim, Germany).

Nucleotide sequence accession numbers

The nucleotide sequences surrounding $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-72}}$ have been deposited in GenBank with the accession number AB828598 and AB857844, respectively.

Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board of the Institute of Medicine, Tribhuvan University (ref. 6-11-E) and the Biosafety Committee, National Center for Global Health and Medicine (approval number: 23-M-49).

Results

Antimicrobial susceptibilities

Four of the 5 isolates were resistant to carbapenems (doripenem, imipenem and meropenem) and piperacillin/tazobactam, whereas the fifth was susceptible to piperacillin/tazobactam, doripenem and meropenem and showed intermediate resistance to imipenem (Table 1). All 5 isolates were highly resistant to cephalosporins (ceftazidime and cefepime), aztreonam, aminoglycosides (arbekacin, amikacin and gentamicin), ciprofloxacin, colistin and fosfomycin, and all 5 showed intermediate resistance to tigecycline. The four isolates resistant to carbapenems were negative with the modified Hodge test, but three of the four isolates were positive with the meropenem-sodium mercaptoacetic acid double-disk synergy test and E-test/EDTA.

Drug-resistant genes

All 5 isolates tested had several genes associated with β-lactam and aminoglycoside-resistance (Table 1). These isolates had $bla_{\text{VEB-1}}$, $bla_{\text{OXA-10}}$, $bla_{\text{TEM-1}}$, $bla_{\text{ADC-67}}$ (*ampC*), *armA* and *aadA1*; 3 had $bla_{\text{NDM-1}}$; and 1 had $bla_{\text{OXA-72}}$. None of these isolates had any other β-lactamase encoding genes, including the class A genes bla_{SHVs} and $bla_{\text{CTX-Ms}}$; the class B genes bla_{AIM}, bla_{DIM}, bla_{FIM}, bla_{GIM}, bla_{IMPs}, bla_{INDs}, bla_{KHM}, bla_{SIM}, bla_{SMB}, bla_{SPM}, bla_{TMBs}, and bla_{VIMs}; or the class D gene bla_{OXAs} except for $bla_{\text{OXA-10}}$ and $bla_{\text{OXA-72}}$. None had other genes encoding 16S rRNA methylases or aminoglycoside acetyl/adenylyltransferases. All 5 isolates had point mutations in the quinolone-resistance-determining regions of *gyrA* and *parC*, with amino acid substitutions of S83I and D87E in GyrA and S80I in ParC, but none had any mutations in the *pmrCAB* operon and *fos* genes. All sequences of the drug-resistant genes tested were identical to those registered in GenBank.

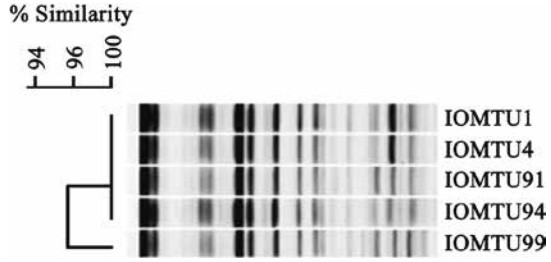

Figure 1 PFGE profiles obtained following *SfiI* digestion of *P. rettgeri* chromosomes.

PFGE and southern hybridyzation

Of the 5 *P. rettgeri* isolates, 4 had identical PFGE patterns and the fifth showed 95.7% similarity (Figure 1). Three of these isolates had a plasmid harboring $bla_{\text{NDM-1}}$ and one had a plasmid harboring $bla_{\text{OXA-72}}$, with plasmid sizes ranging from 9.42 to 23.1 kbp (data not shown).

Genomic structures surrounding $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-72}}$

The genetic environments surrounding $bla_{\text{NDM-1}}$ (Accession no. AB828598) was $bla_{\text{NDM-1}}$-ble_{MBL}-*trpF-dsbC-cutA1*. All 3 isolates harboring $bla_{\text{NDM-1}}$ (IOMTU1, 91 and 99) had the same genetic environments. The $bla_{\text{OXA-72}}$ gene was flanked by conserved inverted repeats at the XerC/XerD binding sites [21], indicating mobilization by site-specific recombination mechanisms. The *rep1* gene was located downstream of $bla_{\text{OXA-72}}$ (Accession no. AB857844).

Discussion

The relatively high MICs to piperacillin/tazobactam and carbapenems of the five *P. rettgeri* isolates were likely due to the presence of $bla_{\text{NDM-1}}$ or $bla_{\text{OXA-72}}$. The enzymatic activities of metallo-β-lactamases, including NDM-1, were not inhibited by tazobactam [22], a β-lactamase inhibitor, in agreement with the MIC profiles of these isolates to piperacillin/tazobactam. The high MICs of all 5 isolates to ceftazidime, cefepime and aztreonam were likely due to the presence of $bla_{\text{VEB-1}}$ [23], and the presence of *armA* in these isolates was likely associated with their extremely high resistance to all aminoglycosides tested [11]. Point mutations in the quinolone-resistance-determining regions of *gyrA* and *parC* have been associated with high resistance to quinolones [24]. Point mutations in *pmrCAB* operon have been associated with the resistance of *Acinetobacter* spp. [25] and *Pseudomonas aeruginosa* [26] to polymxyin and colistin; and the presence of *fos* genes, including *fosA*, *fosA2*, *fosA3*, *fosC* and *fosC2*, has been associated with resistance to fosfomycin in Gram-negative bacteria [27-29].

Plasmids containing $bla_{\text{NDM-1}}$ or $bla_{\text{OXA-72}}$ may be disseminated among Gram-negative pathogens in Nepal. The genetic environments surrounding $bla_{\text{NDM-1}}$ in our *P. rettgeri* strains ($bla_{\text{NDM-1}}$-ble_{MBL}-*trpF-dsbC-cutA1*) were also observed in other plasmids, including *A. baumannii* plasmid pAbNDM-1 from China (Accession no. JN377410), *Citrobacter freundii* plasmid pYE315203 from China (Accession no. JX254913), *E. coli* plasmid pNDM102337 from Canada (Accession no. JF714412), *K. pneumoniae* plasmid pKP-NCGM18-1 from Nepal (Accession no. AB824738) [30], *K. pneumoniae* plasmids pKPX-1, pKPN5047 and pNDM-HN380 from China (Accession nos. AP012055, KC311431 and JX104760, respectively), and *P. rettgeri* plasmid pFR90 (Accession no. JQ362415) from China. In addition, the genetic structures of OXA-72 producing *Acinetobacter* spp [31-34] and *K. pneumoniae* (Accession no. JX268653 and AB825955 deposited in 2012 and 2013, respectively) had the same genetic structure ($bla_{\text{OXA-72}}$-*rep1*) as our strain of *P. rettgeri*.

Conclusions

To our knowledge, this is the first report describing *P. rettgeri* strains harboring $bla_{\text{NDM-1}}$ or $bla_{\text{OXA-72}}$ and *armA* isolated from patients in Nepal. These 5 strains were highly resistant to both β-lactams and aminoglycosides and expanded in a clonal manner in the hospital.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

TT: Performed PCR and sequencing, analyzed data and drafted the manuscript. TMA: Performed entire genome sequencing. RKD and MKS: Performed drug susceptibility tests. HO: Supervised this study. KS: Performed pulsed-field gel electrophoresis and its pattern analysis. TK and BMP: Designed protocols and supervised this study. All authors read and approved the final manuscript.

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Author details

[1]Department of Infectious Diseases, Research Institute, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku, Tokyo 162-8655, Japan. [2]Department of International Medical-Cooporation, National Center for Global Health and Medicine, Shinjuku, Tokyo, Japan. [3]Department of Microbiology, Institute of Medicine, Tribhuvan University, Maharajgunj, Kathmandu, Nepal.

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Table 1
Description of the 23 cefotaxime-resistant *Salmonella enterica* isolates.

Year of isolation	Isolate ID	Serovar	Phage type	Genes encoding cephalosporin resistance	Sex	Age (years)	Travel abroad	Resistance profile
2008	0812M7303	Typhimurium	193	$bla_{CTX-M-55}$	M	50	Thailand	CHL, CIP, FFN, GEN, SUL, STR, TET
	0811R10895	Typhimurium	RDNC	$bla_{CTX-M-1}$	M	1	Unknown	SUL,TET
	0809W37247	Stanley		$bla_{CMY-2-like}$	F	37	No	AMC, CHL, FFN, SUL, STR, TET
	0809F35063	Stanley		$bla_{CMY-2-like}$	F	6	Unknown	AMC, CHL, FFN, GEN, SUL, STR, TET
	0808S63221	Typhimurium	NT	$bla_{CMY-2-like}$	M	20	Thailand	AMC, CHL, FFN, SUL, STR, TET
	0807F21428	Stanley		$bla_{CMY-2-like}$	F	22	Thailand	AMC, CHL, FFN, GEN, SUL, STR, TET
	0806H16365	Stanley		$bla_{CMY-2-like}$	M	2	Unknown	AMC, CHL, FFN, GEN, SUL, STR, TET
	0806R9615	Typhimurium	U292	$bla_{CTX-M-3}$	M	12	No	None
	0805R9530	Typhimurium	NT	$bla_{CTX-M-14}$	M	47	Greece	AMC, CHL, GEN, SUL, STR, TMP
2009	0911W58164	Heidelberg		$bla_{CTX-M-14}$	M	40	Egypt	GEN, SUL, STR
	0910W56953	subsp. enterica (I)		$bla_{CMY-2-like}$	M	55	Thailand	AMC, CHL, CIP, FFN, GEN, NAL, SUL, STR, TET
	0910F48822	Isangi		$bla_{CMY-2-like}$, bla_{OXA-10}	M	<1	South Africa	AMC, CHL, CIP, FFN, GEN, NAL, SUL, STR, TET, TMP
	0909F36769	O:6,8; H:e,h:-		$bla_{CMY-2-like}$	M	49	No	AMC, CHL, FFN, SUL, STR, TET, TMP
	0905W18230	O:4,5,12; H:i:-	U302	$bla_{CTX-M-15}$	M	48	Unknown	CHL, CIP, GEN, SUL, STR, TET, TMP
	0904R11448	Enteritidis	1	$bla_{CTX-M-15}$	F	44	Egypt	GEN
	0904W9384	Typhimurium	193	$bla_{CTX-M-15}$	F	54	No	CHL, CIP, FFN, GEN, SUL, STR, TET
	0903T66197	O:4,5,12; H:i:-	193	$bla_{CTX-M-55}$	F	46	Unknown	GEN, SUL, STR, TET, TMP
2010	1003F13978	O:4,12; H:i: -	193	$bla_{CTX-M-55}$	M	16	Thailand	CHL, CIP, FFN, SUL, STR, TET
	1001M23541	Infantis		$bla_{CTX-M-55}$	F	56	Thailand	CHL, CIP, FFN, GEN, NAL, SUL, STR, TET, TMP
	1010H59657	Senftenberg		$bla_{CTX-M-15}$	M	36	Egypt	SUL, TMP
	1008R13307	Typhimurium	193	$bla_{CTX-M-55}$	F	21	Thailand	CHL, CIP, FFN, GEN, SUL, STR, TET
	1002H3270	Stanley		$bla_{CMY-2-like}$	F	58	Thailand	AMC, CHL, FFN, SUL, STR, TET
	1002W11208	O:4,5,12; H:i:-	193	$bla_{CTX-M-55}$	F	58	Unknown	CHL, CIP, FFN, GEN, SUL, STR, TET

CHL, chloramphenicol; CIP, ciprofloxacin, FFN, florfenicol; GEN, gentamicin; SUL, sulfamethoxazole; STR, streptomycin; TET, tetracycline; AMC, amoxicillin/clavulanic acid; TMP, trimethoprim; NAL, nalidixic acid.

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Mia Torpdahl
Eva Møller Nielsen
Frank Hansen
Steen Ethelberg
Anette M. Hammerum*
Statens Serum Institut, Copenhagen, Denmark

* Corresponding author. Present address: Department of Microbiology and Infection Control, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark.
Tel.: +45 32 68 33 99; fax: +45 32 68 32 31.
E-mail address: ama@ssi.dk (A.M. Hammerum)

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Dissemination of multidrug-resistant *Klebsiella pneumoniae* clinical isolates with various combinations of carbapenemases (NDM-1 and OXA-72) and 16S rRNA methylases (ArmA, RmtC and RmtF) in Nepal

Sir,

The carbapenemases NDM-1 and OXA-72 hydrolyse almost all β-lactams. NDM-1-producing Enterobacteriaceae and OXA-72-producing *Acinetobacter* spp. have been reported in various countries [1,2]. To date, OXA-72-producing isolates of bacterial species other than *Acinetobacter* spp. have not been reported.

Acquired 16S rRNA methylase genes responsible for high-level resistance to various aminoglycosides have been widely distributed among Enterobacteriaceae, including *Klebsiella pneumoniae* and glucose-non-fermentative bacteria [3]. 16S rRNA methylase-producing Gram-negative pathogens have been isolated in various countries [3], including Nepal [4]. The 16S rRNA methylases ArmA and RmtC are widely spread among various bacterial species, including Enterobacteriaceae and *Acinetobacter* spp.

In this study, 25 *K. pneumoniae* isolates were obtained from 25 inpatients during the period May–October 2012 at Tribhuvan University Teaching Hospital (Kathmandu, Nepal), of which 13 isolates were obtained from sputa and 12 were from pus samples. Isolates were identified phenotypically and species identification

Table 1
Summary of the characteristics of the 25 *Klebsiella pneumoniae* strains, including antimicrobial resistance profiles and resistance genes.

Strain	MIC (μg/mL)												Carbapenemases	ESBL	16S rRNA methylases	Mutations in DNA gyrase	
	PIP	TZP	CAZ	CTX	FEP	IPM	MEM	ATM	ABK	AMK	GEN	CIP				*gyrA*	*parC*
IOMTU 23	>1024	512	>1024	1024	256	16	32	512	>1024	>1024	>1024	128	NDM-1, OXA-72	CTX-M-15, SHV-158, TEM-1	RmtC, RmtF	S83I	S80I
IOMTU 25	>1024	1024	>1024	512	128	32	64	512	1024	1024	>1024	128	NDM-1	CTX-M-15, SHV-28, TEM-1	RmtF	S83I	S80I
IOMTU 40	>1024	256	>1024	1024	256	16	32	128	1024	>1024	1024	128	NDM-1	CTXM-15, SHV-28	ArmA	S83F, D87A	S80I
IOMTU 46	>1024	512	>1024	>1024	128	16	32	512	>1024	>1024	>1024	128	NDM-1	CTX-M-15, SHV-11, TEM-1	RmtC, RmtF	S83I	S80I
IOMTU 53	>1024	512	>1024	>1024	512	32	32	512	>1024	>1024	>1024	64	NDM-1, OXA-72	CTX-M-15, SHV-11, TEM-1	RmtC, RmtF	S83I	S80I
IOMTU 67	512	8	64	512	128	<0.5	<0.5	32	1	4	64	32	–	CTX-M-15, SHV-28, TEM-1	–	S83F, D87A	S80I
IOMTU 74	>1024	512	32	256	128	4	8	128	>1024	>1024	>1024	2	NDM-1, OXA-72	CTX-M-15, SHV-1	ArmA	S83F, D87A	S80I
IOMTU 76	>1024	1024	>1024	512	128	32	64	512	>1024	>1024	>1024	64	NDM-1	CTX-M-15, SHV-28, TEM-1	RmtF	S83I	S80I
IOMTU 83	1024	512	128	1024	128	32	32	128	>1024	>1024	>1024	128	NDM-1, OXA-72	CTX-M-15, SHV-28	ArmA	S83F, D87A	S80I
IOMTU 89	>1024	512	>1024	>1024	256	32	32	1024	>1024	>1024	>1024	128	NDM-1	CTX-M-15, SHV-28, TEM-1	RmtF	S83Y, D87F	S80I
IOMTU 100	>1024	1024	>1024	>1024	128	32	64	512	>1024	>1024	>1024	64	NDM-1	CTX-M-15, SHV-28, TEM-1	RmtF	S83F, D87A	S80I
IOMTU 102	>1024	512	>1024	1024	256	32	32	256	512	>1024	>1024	128	NDM-1	CTX-M-15, SHV-28	ArmA	S83F, D87A	S80I
IOMTU 103	>1024	8	64	>1024	64	<0.5	<0.5	128	1	8	32	128	–	CTX-M-15, SHV-28, TEM-1	–	S83F, D87A	S80I
IOMTU 111	>1024	1024	>1024	>1024	512	16	32	512	>1024	>1024	1024	64	NDM-1, OXA-72	CTX-M-15, TEM-1	RmtC	S83I	S80I
IOMTU 116.1	>1024	512	>1024	1024	512	8	8	256	>1024	>1024	512	64	NDM-1	CTX-M-15, SHV-28	ArmA	S83F, D87A	S80I
IOMTU 116.2	>1024	512	>1024	>1024	>1024	8	16	256	>1024	>1024	1024	64	NDM-1	CTX-M-15, SHV-28	ArmA	S83F, D87A	S80I
IOMTU 117	>1024	512	>1024	1024	128	4	4	128	1024	>1024	1024	64	NDM-1	CTX-M-15, SHV-28, TEM-1	ArmA	S83F, D87A	S80I
IOMTU 120	>1024	16	512	>1024	>1024	<0.5	<0.5	1024	>1024	>1024	>1024	128	–	CTX-M-15, SHV-28,TEM-1	RmtF	S83Y, D87N	S80I
IOMTU 122	>1024	512	>1024	>1024	512	16	16	256	>1024	>1024	>1024	128	NDM-1, OXA-72	CTX-M-15, SHV-28	ArmA	S83F, D87A	S80I
IOMTU 125	>1024	4	32	512	256	<0.5	<0.5	64	>1024	>1024	>1024	8	OXA-72	CTX-M-15, SHV-28, TEM-1	ArmA	S83F, D87A	S80I
IOMTU 138	>1024	128	256	>1024	>1024	<0.5	2	512	>1024	>1024	>1024	>1024	OXA-72	CTX-M-15, SHV-11, TEM-1	RmtF	S83F, D87N	No mutation
IOMTU 139	>1024	128	128	512	256	2	2	512	32	32	64	256	–	CTX-M-15, SHV-28, TEM-1	–	S83Y, D87G	S80I
IOMTU 145	>1024	8	32	1024	256	<0.5	<0.5	64	4	4	<0.5	2	–	CTX-M-15, TEM-1	–	No mutation	No mutation
IOMTU 154	>1024	1024	>1024	1024	256	16	32	64	>1024	>1024	1024	16	NDM-1, OXA-72	CTX-M-15, SHV-28, TEM-1	RmtC	S83F, D87A	S80I
IOMTU 164	>1024	8	64	512	256	<0.5	<0.5	64	4	4	<0.5	<0.5	–	CTX-M-15, SHV-83, TEM-1	–	No mutation	No mutation

MIC, minimum inhibitory concentration; PIP, piperacillin; TZP, piperacillin/tazobactam; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; IPM, imipenem; MEM, meropenem; ATM, aztreonam; ABK, arbekacin; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; ESBL, extended-spectrum β-lactamase.

was confirmed by 16S rRNA sequencing. Minimum inhibitory concentrations of antibiotics were determined by the microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M07-A9.

Entire genomes of the isolates were sequenced by MiSeq™ (Illumina, San Diego, CA). CLC Genomics Workbench v.5.5 (CLC bio, Tokyo, Japan) was used to search 923 drug resistance genes, including genes encoding β-lactamases, 16S rRNA methylases and aminoglycoside-acetyl/adenyltransferases, as well as point mutations in *gyrA* and *parC* associated with quinolone resistance. The genetic environments surrounding bla_{NDM-1}, bla_{OXA-72} and 16S rRNA methylase-encoding genes were determined. Multilocus sequence typing (MLST) and clonal complexes (CCs) were determined according to the *K. pneumoniae* MLST database website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) and eBURST v.3 (http://eburst.mlst.net), respectively.

Pulsed-field gel electrophoresis (PFGE) analysis was performed and fingerprinting patterns were analysed by the unweighted pair-group method.

All isolates were resistant to piperacillin, of which 19 isolates were resistant to piperacillin/tazobactam. All isolates were resistant to ceftazidime, cefotaxime and cefepime. Seventeen isolates were resistant to carbapenems (imipenem and meropenem). All isolates are resistant to aztreonam. Twenty isolates were resistant to all aminoglycosides tested (arbekacin, amikacin and gentamicin). Twenty-two isolates were resistant to ciprofloxacin (Table 1).

The majority of isolates had various combinations of genes encoding carbapenemases (bla_{NDM-1} and bla_{OXA-72}) and 16S rRNA methylases (*armA*, *rmtC* and *rmtF*) (Table 1). These isolates also had extended-spectrum β-lactamase-encoding genes, including $bla_{CTX-M-15}$, bla_{TEM-1} and/or bla_{SHV}-type, as well as aminoglycoside-modifying enzymes, including *aac(6′)-Ib* and/or *aadA2*. Twenty-three isolates had two or three point mutations in the quinolone resistance-determining regions of *gyrA* and *parC*.

The genetic environment surrounding bla_{NDM-1} (GenBank accession no. AB824738) including *rmtC* was a unique structure, which was *orf1–tniB–orf2–orf3–rmtC–*bla_{NDM-1}*–*ble_{MBL}*–trpF–dsbC–cutA1–qroL*. The genetic environment surrounding *armA* (GenBank accession no. AB825954) from nucleotides 19 to 14 138 had >99.9% sequence identity to a nucleotide sequence from nucleotides 65 492 to 79 611 of the plasmid pCTX-M3 (GenBank accession no. AF550415). The genetic environment surrounding *rmtF* (GenBank accession no. AB824739) from nucleotides 268 to 9812 had >99.9% sequence identity to a nucleotide sequence from nucleotides 49 291 to 58 835 of the plasmid pKPX-1 (GenBank accession no. AP012055). The genetic environment surrounding bla_{OXA-72} (GenBank accession no. AB825955) from nucleotides 1 to 8970 was identical to that of pAB-NCGM253 (GenBank accession no. AB823544).

The clinical isolates of *K. pneumoniae* tested belonged to one of the following sequence types (STs): ST11; ST14; ST15; ST29; ST43; ST340; ST378; ST395; ST437; ST1231; and ST1232. Of these isolates, 14 belonged to CC14 and 5 belonged to CC11. These results mostly corresponded with the results of PFGE pattern analysis, which revealed two clusters showing >60% similarity (clusters I and II). Cluster I comprised 12 isolates belonging to CC14 and cluster II comprised 4 isolates belonging to CC11.

NDM-1-producers have epidemiological links to the Indian subcontinent as of 2011 [5]. There was, nevertheless, no report of NDM-1-producers in Nepal. We recently found NDM-1-producing *Pseudomonas aeruginosa* and a novel variant NDM-8-producing *Escherichia coli* isolates in Nepal [4].

This is the first report describing OXA-72-producers in South Asia, suggesting that OXA-72-producers have disseminated in this region. This is also the first report of OXA-72-producing *K. pneumoniae* clinical isolates. Up to now, OXA-72-producers were reported to be only *Acinetobacter* spp.

The present study suggests that aminoglycoside-resistant Gram-negative pathogens producing ArmA, RmtC and RmtF disseminated in medical settings in Nepal. These pathogens producing 16S rRNA methylases may also disseminate in neighbouring countries. Hidalgo et al. [6] recently reported that 14% of Enterobacteriaceae isolates from an Indian hospital had 16S rRNA methylases, of which 24% produced RmtF.

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Tatsuya Tada
Tohru Miyoshi-Akiyama
Department of Infectious Diseases, Research Institute, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku, Tokyo 162-8655, Japan

Rajan K. Dahal
Shyam K. Mishra
Department of Microbiology, Institute of Medicine, Tribhuvan University, Maharajgunj, Kathmandu, Nepal

Hiroshi Ohara
Department of International Medical Co-operation, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku, Tokyo, Japan

Kayo Shimada
Teruo Kirikae*
Department of Infectious Diseases, Research Institute, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku, Tokyo 162-8655, Japan

Bharat M. Pokhrel
Department of Microbiology, Institute of Medicine, Tribhuvan University, Maharajgunj, Kathmandu, Nepal

* Corresponding author. Tel.: +81 3 3202 7181x2838; fax: +81 3 3202 7364.
E-mail address: tkirikae@ri.ncgm.go.jp (T. Kirikae)

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NDM-12, a Novel New Delhi Metallo-β-Lactamase Variant from a Carbapenem-Resistant *Escherichia coli* Clinical Isolate in Nepal

Tatsuya Tada,[a] Basudha Shrestha,[c] Tohru Miyoshi-Akiyama,[a] Kayo Shimada,[a] Hiroshi Ohara,[b] Teruo Kirikae,[a] Bharat M. Pokhrel[c]

Department of Infectious Diseases, Research Institute,[a] and Department of International Medical-Cooperation,[b] National Center for Global Health and Medicine, Shinjuku, Tokyo, Japan; Department of Microbiology, Institute of Medicine, Tribhuvan University, Maharajgunj, Kathmandu, Nepal[c]

A novel New Delhi metallo-β-lactamase variant, NDM-12, was identified in a carbapenem-resistant *Escherichia coli* clinical isolate obtained from a urine sample from a patient in Nepal. NDM-12 differed from NDM-1 by two amino acid substitutions (M154L and G222D). The enzymatic activities of NDM-12 against β-lactams were similar to those of NDM-1, although NDM-12 showed lower k_{cat}/K_m ratios for all β-lactams tested except doripenem. The bla_{NDM-12} gene was located in a plasmid of 160 kb.

Metallo-β-lactamases (MBLs) usually confer reduced susceptibility to carbapenems, cephalosporins, and penicillins but not monobactams (1). Acquired MBLs are produced by Gram-negative bacteria, including *Acinetobacer* spp., *Pseudomonas aeruginosa*, and several *Enterobacteriaceae* (1). MBLs are categorized by their amino acid sequences into various types (2–4), including AIM (5), DIM (6), FIM (7), GIM (8), IMPs (9), KHM (10), NDMs (11), SMB (12), SIM (13), SPM (14), TMBs (15), and VIMs (16). The most prevalent types of MBLs are IMP-, VIM-, and NDM-type enzymes (1, 2, 17). NDM-1 was initially isolated from *Klebsiella pneumoniae* and *Escherichia coli* in 2008 in Sweden (11). Subsequently, at least 11 NDM variants (www.lahey.org/studies) have been reported in several countries (4, 18–29).

This study was ethically reviewed and approved by the Institutional Review Board of the Institute of Medicine at Tribhuvan University (reference 6-11-E) and the Biosafety Committee at the National Center for Global Health and Medicine (approval no. 26-D-088 and 26-D-089).

E. coli IOMTU388.1 was isolated from a urine sample obtained from a patient in 2013 in a university hospital in Nepal. The isolate was phenotypically identified, and the species identification was confirmed by 16S rRNA sequencing (30). *E. coli* DH5α (TaKaRa Bio, Shiga, Japan) and *E. coli* BL21-CodonPlus(DE3)-RIP (Agilent Technologies, Santa Clara, CA) were used as hosts for recombinant plasmids and for expression of bla_{NDM-1} and bla_{NDM-12}, respectively.

MICs were determined using the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (31). The MICs of β-lactams for *E. coli* IOMTU388.1 are shown in Table 1, and the MICs of other antibiotics were as follows: amikacin, >1,024 μg/ml; arbekacin, >1,024 μg/ml; ciprofloxacin, 128 μg/ml; colistin, ≤0.125 μg/ml; fosfomycin, 8 μg/ml; gentamicin, >1,024 μg/ml; kanamycin, >1,024 μg/ml; levofloxacin, 32 μg/ml; minocycline, 8 μg/ml; tigecycline, ≤0.125 μg/ml; and tobramycin, >1,024 μg/ml. PCR analysis was performed to detect the MBL genes bla_{DIM}, bla_{GIM}, bla_{IMP}, bla_{NDM}, bla_{SIM}, bla_{SPM}, and bla_{VIM} (32, 33). The isolate was PCR positive for bla_{NDM} but negative for the other MBL genes tested. The DNA sequence of the PCR product revealed that the isolate had bla_{NDM-12}. Multilocus sequence typing (MLST) of IOMTU388.1 typed it as ST635 (*E. coli* MLST Database; http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi.html). bla_{NDM-1} obtained from *P. aeruginosa* IOMTU9 (29) was used as a reference gene.

The bla_{NDM-12} sequence had 2 amino acid substitutions (M154L and G222D) compared with bla_{NDM-1} (accession no. JF798502) and one substitution (G222D) compared with NDM-4 (accession no. JQ348841).

The bla_{NDM-1} and bla_{NDM-12} genes were cloned into the corresponding sites of pHSG398 (TaKaRa, Shiga, Japan) using the primer set EcoRI-NDM-F (5′-GGGAATTCATGGAATTGCCCAATATTATG-3′) and PstI-NDM-R (5′-AACTGCAGTCAGCGCAGCTTGTCGGCCAT-3′). *E. coli* DH5α was transformed with pHSG398-NDM-1 or pHSG398-NDM-12.

The open reading frames of NDM-1 and NDM-12 without signal peptide regions were cloned into the pET28a expression vector (Novagen, Inc., Madison, WI) using the primer set BamHI-TEV-NDM-F (5′-ATGGATCCGAAAACCTGTATTTCCAAGGCCAGCAAATGGAAACTGGCGAC-3′) and XhoI-NDM-R (5′-ATCTCGAGTCAGCGCAGCTTGTCGGCCATG-3′). The resulting plasmids were transformed into *E. coli* BL21-CodonPlus(DE3)-RIP (Agilent Technologies, Santa Clara, CA). Both recombinant NDM-1 and NDM-12 were purified simultaneously using Ni-nitrilotriacetic acid (NTA) agarose according to the manufacturer's instruction (Qiagen, Hilden, Germany). His tags were removed by digestion with TurboTEV protease (Accelagen, San Diego, CA) and untagged proteins were purified by an additional passage over the Ni-NTA agarose. The purities of NDM-1 and NDM-12, which were estimated by SDS-PAGE, were greater than 90%. During the purification procedure, the presence of β-lactamase activity was monitored using nitrocefin (Oxoid, Ltd., Basingstoke, United Kingdom). Initial hydrolysis rates were determined in 50 mM Tris-HCl buffer (pH 7.4) containing 0.3 M NaCl and 5 μM $Zn(NO_3)_2$ at 37°C, using a UV-visible spectrophotometer (V-530; Jasco, Tokyo, Japan). The K_m and k_{cat} values and the k_{cat}/K_m ratio were determined by analyzing β-lactam hydrolysis with a Lineweaver-Burk plot. Wavelengths and extinction coefficients for

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Address correspondence to Teruo Kirikae, tkirikae@ri.imcj.go.jp.
T.T. and B.S. contributed equally to this article.

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TABLE 1 MICs of various β-lactams for *E. coli* IOMTU388.1 and *E. coli* DH5α transformed with plasmids encoding NDM-12 or NDM-1

Antibiotic(s)	MIC (μg/ml) for strain:			
	IOMTU388.1	DH5α(pHSG398/NDM-1)	DH5α(pHSG398/NDM-12)	DH5α(pHSG398)
Ampicillin	>1,024	256	512	4
Ampicillin-sulbactam	>1,024	128	128	2
Aztreonam	64	≤0.063	≤0.063	≤0.063
Cefepime	512	0.5	1	≤0.063
Cefoselis	1,024	16	8	1
Cefotaxime	>1,024	8	16	≤0.063
Cefoxitin	>1,024	64	16	≤0.063
Cefpirome	512	2	2	≤0.063
Ceftazidime	>1,024	256	256	≤0.063
Ceftriaxone	>1,024	16	16	≤0.063
Cefradine	>1,024	512	256	16
Doripenem	32	0.063	0.063	≤0.063
Imipenem	16	0.5	0.25	≤0.063
Meropenem	64	0.25	0.125	≤0.063
Moxalactam	>1,024	16	4	0.125
Penicillin G	>1,024	256	256	32

β-lactam substrates have been reported previously (34–36). The K_m and k_{cat} values (means ± standard deviations) were obtained from three individual experiments. The enzymatic activities of NDM-1 were measured in parallel with those of NDM-12.

The plasmid harboring bla_{NDM-12} was extracted (37) and sequenced using MiSeq (Illumina, San Diego, CA). The size of the plasmid harboring bla_{NDM-12} was determined using pulsed-field gel electrophoresis (PFGE) and Southern hybridization. A probe for bla_{NDM-12} from IOMTU388.1 was amplified by PCR using the primer sets for EcoRI-NDM-F and PstI-NDM-R. Signal detection was carried out using the digoxigenin (DIG) High Prime DNA labeling and detection starter kit II (Roche Applied Science, Indianapolis, IN).

Mating-out assays between the parental strain IOMTU388.1 and the chloramphenicol-resistant *E. coli* strain BL21 were performed in LB broth using a 1:4 donor/recipient ratio for 3 h at 37°C. Transconjugants were selected on Muller-Hinton agar plates containing ceftazidime (100 μg/ml) and chloramphenicol (30 μg/ml). Selected transconjugants harboring bla_{NDM-12} were confirmed by PCR with the primer set EcoRI-NDM-F and PstI-NDM-R.

E. coli DH5α harboring bla_{NDM-1} or bla_{NDM-12} showed reduced susceptibility to moxalactam and all penicillins, cephalosporins, and carbapenems tested compared with DH5α harboring a vector control (Table 1). The MICs of the β-lactams cefoxitin and moxalactam for DH5α harboring bla_{NDM-12} were 4-fold less than those for DH5α harboring bla_{NDM-1} (Table 1).

As shown in Table 2, recombinant NDM-1 and NDM-12 hydrolyzed all β-lactams tested except for aztreonam. The profiles of enzymatic activities of NDM-12 against β-lactams tested were similar to those of NDM-1, although NDM-12 had lower k_{cat}/K_m ratios for all β-lactams tested except for doripenem. The lower k_{cat}/K_m ratios were likely to be caused by the lower k_{cat} values of NDM-12 compared with those of NDM-1, as the values of NDM-12 were 11.4 to 73.6% of those of NDM-1 (Table 2). The profiles of enzymatic activities of NDM-1 except for cefoxitin were similar to those of NDM-1 that we reported previously (29). The k_{cat}/K_m ratio for cefoxitin in Table 2 was 10-fold higher than that

TABLE 2 Kinetic parameters of the NDM-1 and NDM-12 enzymes[a]

β-Lactam	NDM-1[b]			NDM-12[b]		
	K_m (μM)	k_{cat} (s^{-1})[b]	k_{cat}/K_m (μM^{-1} s^{-1})	K_m (μM)	k_{cat} (s^{-1})[b]	k_{cat}/K_m (μM^{-1} s^{-1})
Ampicillin	231 ± 33	249 ± 22	1.1	126 ± 4	136 ± 2	1.1
Aztreonam	NH[c]	NH	NH	NH	NH	NH
Cefepime	162 ± 7	31 ± 1	0.19	103 ± 6	11.1 ± 0.2	0.11
Cefotaxime	102 ± 16	137 ± 7	1.1	45 ± 4	38 ± 1	0.84
Cefoxitin	13 ± 1	6.7 ± 0.1	0.50	26 ± 2	0.66 ± 0.01	0.02
Ceftazidime	202 ± 7	56 ± 1	0.28	53 ± 4	5.7 ± 0.1	0.11
Cefradine	27 ± 3	72 ± 1	2.7	57 ± 4	16 ± 1	0.28
Doripenem	201 ± 27	114 ± 9	0.57	88 ± 2	53 ± 1	0.60
Imipenem	249 ± 43	44 ± 2	0.34	125 ± 22	22 ± 2	0.18
Meropenem	81 ± 10	139 ± 10	1.7	91 ± 8	53 ± 2	0.58
Moxalactam	4.5 ± 2.3	7.6 ± 0.3	2.0	67 ± 5	6.0 ± 0.2	0.09
Penicillin G	67 ± 6	104 ± 1	1.6	64 ± 8	42 ± 2	0.66

[a] The proteins were initially modified by a His tag, which was removed after purification.
[b] The K_m and k_{cat} values shown represent the means from 3 independent experiments ± standard deviations.
[c] NH, no hydrolysis was detected under conditions with substrate concentrations up to 1 mM and enzyme concentrations up to 700 nM.

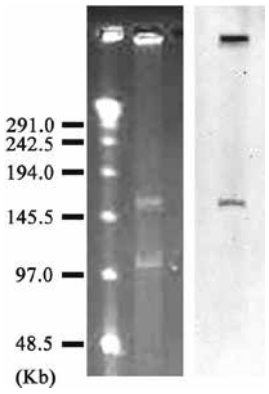

FIG 1 Localization of bla_{NDM-12} on a plasmid from *E. coli* strain IOMTU388.1 separated by PFGE. Left lane, MidRange PFG marker (New England BioLabs, Tokyo, Japan); middle lane, plasmids from *E. coli* strain IOMTU388.1; right lane, hybridization of the plasmid with a probe specific for bla_{NDM-12}.

in our previous study (see Table 2 in reference 29). The difference between the ratios may be explained by the use of different buffer solutions in the kinetics assays (Tris buffer and phosphate buffer, respectively). It was reported that phosphate ions affected the enzymatic activities of metallo-β-lactamase IMP-1 (38). Phosphate ions may affect the enzymatic activities of NDM-1 against cefoxitin.

The MBL gene bla_{NDM-12} in *E. coli* IOMTU388.1 was detected in a plasmid, pIOMTU388-NDM (accession no. AB926431), with a size of 160 kb (Fig. 1). The sequence surrounding bla_{NDM-12} was bla_{NDM-12}-ble_{MBL}-*trpF-dsbC-tnpA-sulI-qacE*Δ*I*. This plasmid showed more than 99.9% identity at the nucleotide sequence level to the sequence located from bp 70978 to 77904 in the pGUE-NDM plasmid (accession no. JQ364967) from *E. coli* strain GUE, which was isolated in India (39), and also showed 99.9% identity at the nucleotide sequence level to the sequence located from bp 372 to 7298 in the pEC77-NDM plasmid (accession no.AB898038) from *E. coli* strain NCGM77, which was isolated in Japan (40). The plasmid harboring bla_{NDM-12} belonged to the IncF incompatibility group and was conjugated from IOMTU388.1 to *E. coli* BL21 at a conjugative frequency of 1.63×10^{-3}.

The 2 substitutions M154L and G222D in NDM-12 (compared with NDM-1) affected the activity of the enzyme (Table 2). Nordmann et al. (24) reported that a mutant containing M154L (NDM-4) possessed increased hydrolytic activity toward carbapenems and several cephalosporins compared to NDM-1. Unexpectedly, NDM-12, which contains the M154L substitution, did not show an increase in hydrolytic activities. The substitution at position 222 found in NDM-12 has been not reported in other variants, to our knowledge. Although we did not directly compare the enzymatic activity of NDM-12 with those of NDM-4, the substitution of G222D in NDM-12 may be associated with a decrease in hydrolytic activities toward these antibiotics (Table 2). Position 222 is located in loop L10 of NDM-1, which forms the active site of NDM-1 with L3 at the bottom of a shallow groove (41–44). Among all known 11 NDM-1 variants, amino acid substitutions were found at 13 amino acid positions, including positions 28, 32, 36, 69, 74, 88, 95, 130, 152, 154, 200, and 233. Positions 28, 32, and 36 were in the signal peptide region. Positions 95, 130, and 154 have been reported to affect β-lactam-hydrolyzing activities, although whether the activities are affected by the other 6 substitutions has not been reported. Residue 95 is located in α1 on the protein surface, and the amino acid substitution at position 95 affected the k_{cat} values of NDM-3 (40). The substitution at position 130 (Met to Leu) showed increased hydrolytic activity toward carbapenems and several cephalosporins compared to NDM-1 (24, 29).

This is the first report describing NDM-12-producing *E. coli* in Nepal. NDMs seem to evolve rapidly; therefore, careful monitoring of NDM-producing pathogens is required.

Nucleotide sequence accession number. The plasmid sequence including bla_{NDM-12} has been deposited in GenBank under accession no. AB926431.

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Identification of a Novel 6′-*N*-Aminoglycoside Acetyltransferase, AAC(6′)-Iak, from a Multidrug-Resistant Clinical Isolate of *Stenotrophomonas maltophilia*

Tatsuya Tada,[a] Tohru Miyoshi-Akiyama,[a] Rajan K. Dahal,[c] Shyam K. Mishra,[c] Kayo Shimada,[a] Hiroshi Ohara,[b] Teruo Kirikae,[a] Bharat M. Pokhrel[c]

Department of Infectious Diseases, Research Institute,[a] and Department of International Medical Cooperation,[b] National Center for Global Health and Medicine, Shinjuku, Tokyo, Japan; Department of Microbiology, Institute of Medicine, Tribhuvan University, Maharajgunj, Kathmandu, Nepal[c]

***Stenotrophomonas maltophilia* IOMTU250 has a novel 6′-*N*-aminoglycoside acetyltransferase-encoding gene, *aac(6′)-Iak*. The encoded protein, AAC(6′)-Iak, consists of 153 amino acids and has 86.3% identity to AAC(6′)-Iz. *Escherichia coli* transformed with a plasmid containing *aac(6′)-Iak* exhibited decreased susceptibility to arbekacin, dibekacin, neomycin, netilmicin, sisomicin, and tobramycin. Thin-layer chromatography showed that AAC(6′)-Iak acetylated amikacin, arbekacin, dibekacin, isepamicin, kanamycin, neomycin, netilmicin, sisomicin, and tobramycin but not apramycin, gentamicin, or lividomycin.**

Stenotrophomonas maltophilia is a globally emerging multidrug-resistant Gram-negative pathogen that is most commonly associated with respiratory infections in humans (1) and causes an increasing number of nosocomial respiratory tract and bloodstream infections in immunocompromised patients. *S. maltophilia* exhibits resistance to a broad spectrum of antibiotics, namely, β-lactam antibiotics, macrolides, cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, chloramphenicol, tetracyclines, and polymyxins (1). Several intrinsic antibiotic resistance traits in *S. maltophilia* are known; an increase in membrane permeability and the presence of chromosomally encoded multidrug resistance efflux pumps have also been observed (2).

Aminoglycoside-resistant mechanisms involve primarily aminoglycoside-modifying enzymes (3) and 16S rRNA methylases (4). The 6′-*N*-aminoglycoside acetyltransferases [AAC(6′)s] are of particular interest because they can modify a number of clinically important aminoglycosides. There are two main AAC(6′) subclasses, which differ in their activities against amikacin and gentamicin. The AAC(6′)-I-type enzymes effectively acetylate amikacin but not gentamicin, whereas the AAC(6′)-II-type enzymes effectively acetylate gentamicin but not amikacin (5). To date, 45 genes encoding AAC(6′)-I types, designated *aac(6′)-Ia* to *-Iaj*, have been cloned, and their bacteriological or biochemical properties have been characterized (5–8).

S. maltophilia IOMTU250 was isolated from the endotracheal tube of a patient in a medical ward of a hospital in Nepal in 2012. *Escherichia coli* DH5α (TaKaRa Bio, Shiga, Japan) and *Escherichia coli* BL21-CodonPlus (DE3)-RIP (Agilent Technologies, Santa Clara, CA) were used as hosts for recombinant plasmids and protein expression, respectively. MICs were determined using the microdilution method (9). The MICs of tested aminoglycosides for *S. maltophilia* IOMTU250 are shown in Table 1. The MICs of other antibiotics were as follows: ampicillin, >1,024 μg/ml; ampicillin-sulbactam, 128 μg/ml; aztreonam, 128 μg/ml; ceftazidime, 8 μg/ml; cephradine, 1,024 μg/ml; cefepime, 64 μg/ml; cefotaxime, 64 μg/ml; cefoxitin, 512 μg/ml; chloramphenicol, 8 μg/ml; colistin, 32 μg/ml; fosfomycin, 128 μg/ml; imipenem, 256 μg/ml; levofloxacin, 1 μg/ml; meropenem, 64 μg/ml; minocycline, ≤0.25 μg/ml; penicillin, 512 μg/ml; ticarcillin-clavulanate, 8 μg/ml; tigecycline, ≤0.25 μg/ml; and trimethoprim-sulfamethoxazole, 4 μg/ml.

Genomic DNA was extracted from *S. maltophilia* IOMTU250 using DNeasy blood and tissue kits (Qiagen, Tokyo, Japan) and sequenced with a MiSeq system (Illumina, San Diego, CA). More than 20-fold coverage was achieved. A new 6′-*N*-aminoglycoside acetyltransferase variant was designated *aac(6′)-Iak*.

A synthetic *aac(6′)-Iz* gene (462 bp) was produced by Funako-

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Address correspondence to Teruo Kirikae, tkirikae@ri.imcj.go.jp.

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TABLE 1 MICs of various aminoglycosides for *S. maltophilia* IOMTU250 and *E. coli* strains transformed with *aac(6′)-Iak* and *aac(6′)-Iz*

Strain[a]	MIC[b] (μg/ml)											
	ABK	AMK	APR	DIB	GEN	ISP	KAN	LIV	NEO	NET	SIS	TOB
S. maltophilia IOMTU250	512	64	>512	512	32	64	128	>512	512	512	64	64
E. coli DH5α/pSTV28	0.5	0.25	1	0.25	0.25	0.25	1	2	0.5	0.25	0.25	0.5
E. coli DH5α/pSTV28-*aac(6′)-Iak*	2	1	1	16	0.25	0.5	2	2	4	1	2	4
E. coli DH5α/pSTV28-*aac(6′)-Iz*	4	2	1	16	0.25	0.5	2	2	4	8	2	16

[a] The MICs for *S. maltophilia* and *E. coli* strains were determined with Mueller-Hinton broth preparations and individual aminoglycosides.
[b] ABK, arbekacin; AMK, amikacin; APR, apramycin; DIB, dibekacin; GEN, gentamicin; ISP, isepamicin; KAN, kanamycin; LIV, lividomycin; NEO, neomycin; NET, netilmicin; SIS, sisomicin; TOB, tobramycin.

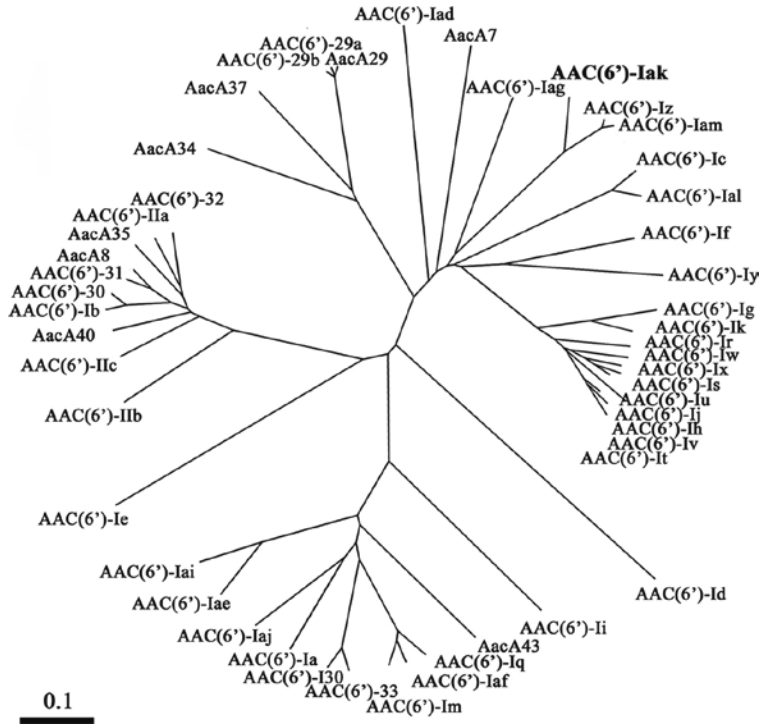

FIG 1 Dendrogram of 6′-*N*-aminoglycoside acetyltransferases [AAC(6′)s] for comparison with AAC(6′)-Iak. The dendrogram was calculated using the CLUSTAL W2 program. Branch lengths correspond to numbers of amino acid exchanges for the proteins. EMBL/GenBank/DDBJ accession numbers of the proteins are as follows: AAC(6′)-Ia, M18967-1; AAC(6′)-Ib, M23634; AAC(6′)-Ic, M94066; AAC(6′)-Id, X12618; AAC(6′)-Ie, M13771; AAC(6′)-If, X55353; AAC(6′)-Ig, L09246; AAC(6′)-Ih, L29044; AAC(6′)-Ii, L12710-1; AAC(6′)-Ij, L29045; AAC(6′)-Ik, L29510; AAC(6′)-Im, CAA91010; AAC(6′)-Iq, AF047556-1; AAC(6′)-Ir, AF031326; AAC(6′)-Is, AF031327; AAC(6′)-It, AF031328; AAC(6′)-Iu, AF031329; AAC(6′)-Iv, AF031330; AAC(6′)-Iw, AF031331; AAC(6′)-Ix, AF031332; AAC(6′)-Iy, AF144880; AAC(6′)-Iz, AF140221; AAC(6′)-Iad, AB119105; AAC(6′)-Iae, AB104852; AAC(6′)-Iaf, AB462903; AAC(6′)-Iag, AB472901; AAC(6′)-Iai, EU886977; AAC(6′)-Iaj, AB709942; AAC(6′)-Iak, AB894482; AAC(6′)-Ial, AB871481; AAC(6′)-Iam, AB971834; AAC(6′)-IIa, M29695; AAC(6′)-IIb, L06163; AAC(6′)-IIc, AF162771; AAC(6′)-29a, AF263519; AAC(6′)-29b, AF263519; AAC(6′)-30, AJ584652; AAC(6′)-31, AJ640197; AAC(6′)-32, EF614235; AAC(6′)-33, GQ337064; AAC(6′)-I30, AY289608; AacA7, U13880; AacA8, AY444814; AacA29, AY139599; AacA34, AY553333; AacA35, AJ628983; AacA37, DQ302723; AacA40, EU912537; and AacA43, HQ247816.

shi Co. Ltd. (Tokyo, Japan). The *aac(6′)-Iak* and *aac(6′)-Iz* genes were cloned into the corresponding sites of pSTV28 using primers SalI-*aac(6′)-Iak*-F (5′-ATGCGTCGACATGACCGGCAGCGCG GCCACGATCCGCCCG-3′) and PstI-*aac(6′)-Iak*-R (5′-ATCTG CAGTCACGCCGATGGCTCCAGTGGCATGCGGAAA-3′) and SalI-*aac(6′)-Iz*-F (5′-ATGGTCGACATGATCGCCAGCGCGCC CACGATCCGCC-3′) and PstI-*aac(6′)-Iz*-R (5′-ATCTGCAGTC ACGCCGATGGCTCCAGCGGCATGCGGAA-3′), respectively. *E. coli* DH5α was transformed with pSTV28-*aac(6′)-Iak* or pSTV28-*aac(6′)-Iz* to assess aminoglycoside resistance.

The open reading frame of AAC(6′)-Iak was cloned into the pQE2 expression vector using the primers SalI-*aac(6′)-Iak*-F and PstI-*aac(6′)-Iak*-R for protein expression. Purification of the recombinant AAC(6′)-Iak protein and thin-layer chromatography (TLC) analysis were performed as previously described (7). The kinetic activities of AAC(6′)-Iak were determined as previously described (8).

AAC(6′)-Iak consists of 153 amino acids. As shown by the dendrograms of AAC(6′) based on amino acid sequences in Fig. 1, AAC(6′)-Iak is close to AAC(6′)-Iz and AAC(6′)-Iam (accession no. AB971834) from *S. maltophilia* (10). Multiple sequence alignments among AAC(6′) enzymes revealed that AAC(6′)-Iak had 86.3% identity to AAC(6′)-Iz from *S. maltophilia* (11), 84.3% identity to AAC(6′)-Iam from *S. maltophilia* (10), 47.7% identity to AAC(6′)-Iag from *Pseudomonas aeruginosa* (8), 43.1% identity to AAC(6′)-If from *Enterobacter cloacae* (12), and 42.8% identity to AAC(6′)-Iy from *Salmonella enterica* (13).

We compared the enzymatic properties of AAC(6′)-Iak with those of AAC(6′)-Iz because they had similar amino acid sequences and were detected in *S. maltophilia*. As shown in Table 1, *E. coli* expressing AAC(6′)-Iak or AAC(6′)-Iz showed decreased susceptibility to all aminoglycosides tested except for apramycin, gentamicin, and lividomycin. The MICs of netilmicin and tobramycin for *E. coli* expressing AAC(6′)-Iak were significantly lower than those for *E. coli* expressing AAC(6′)-Iz. The MICs of the other aminoglycosides tested were not significantly different between *E. coli* isolates expressing AAC(6′)-Iak and AAC(6′)-Iz (Table 1).

S. maltophilia IOMTU250 was highly resistant to all aminoglycosides tested, whereas *E. coli* expressing *aac(6′)-Iak* was susceptible to all aminoglycosides tested except for dibekacin (Table 1). The discrepancy of aminoglycoside susceptibilities between *S. maltophilia* and *E. coli* could be explained by the presence of efflux pump genes specific for *S. maltophilia*. Whole-genome sequencing with the MiSeq system in this study showed that IOMTU250 had the efflux pump genes specific for *S. maltophilia*, including

TABLE 2 Kinetic parameters of AAC(6′)-Iak and AAC(6′)-Iz enzymes[a]

Aminoglycoside[b]	AAC(6′)-Iak			AAC(6′)-Iz		
	K_m (μM)[c]	k_{cat} (s^{-1})[c]	k_{cat}/K_m ($\mu M^{-1}\ s^{-1}$)	K_m (μM)[c]	k_{cat} (s^{-1})[c]	k_{cat}/K_m ($\mu M^{-1}\ s^{-1}$)
ABK	36 ± 13	0.57 ± 0.10	0.016	17 ± 6	0.57 ± 0.06	0.036
AMK	32 ± 17	0.11 ± 0.03	0.004	24 ± 5	0.26 ± 0.02	0.011
DIB	24 ± 3	0.25 ± 0.04	0.010	31 ± 5	0.46 ± 0.05	0.015
ISP	44 ± 9	0.05 ± 0.01	0.001	49 ± 8	0.11 ± 0.01	0.002
KAN	30 ± 7	0.04 ± 0.01	0.001	44 ± 9	0.05 ± 0.01	0.001
NEO	10 ± 2	0.32 ± 0.01	0.034	13 ± 2	0.29 ± 0.01	0.023
NET	70 ± 6	0.30 ± 0.02	0.004	25 ± 8	0.34 ± 0.05	0.013
SIS	4 ± 1	0.16 ± 0.01	0.038	7 ± 1	0.20 ± 0.03	0.028
TOB	16 ± 4	0.08 ± 0.01	0.006	12 ± 2	0.25 ± 0.03	0.021

[a] The proteins were initially modified by a His tag, which was removed after purification.
[b] ABK, arbekacin; AMK, amikacin; DIB, dibekacin; ISP, isepamicin; KAN, kanamycin; NEO, neomycin; NET, netilmicin; SIS, sisomicin; TOB, tobramycin.
[c] K_m and k_{cat} values represent the means of results from 3 independent experiments ± standard deviations.

smeABC, *smeDEF*, *smeZ*, *smeJK*, and the *pcm-tolCsm* operon. These genes are known to be associated with aminoglycoside resistance (14–16), although it is difficult to clarify whether or not these efflux pump genes contribute to aminoglycoside resistance together with *aac(6′)-Iak* in *S. maltophilia* IOMTU250.

As shown in Table 2, recombinanttk;1 AAC(6′)-Iak and AAC(6′)-Iz acetylated arbekacin, amikacin, dibekacin, isepamicin, kanamycin, neomycin, netilmicin, sisomicin, and tobramycin. The profile of enzymatic activities of AAC(6′)-Iak were similar to those of AAC(6′)-Iz, although AAC(6′)-Iak had higher k_{cat}/K_m ratios for neomycin and sisomicin (Table 2).

To examine the acetyltransferase activity of AAC(6′)-Iak against aminoglycosides, we performed TLC using the purified recombinant AAC(6′)-Iak. Lividomycin was used as a negative control because it has a hydroxyl group instead of an amino group at the 6′ position and therefore cannot be acetylated by AAC(6′). As shown in Fig. 2, all aminoglycosides tested except for apramycin, gentamicin, and lividomycin were acetylated by AAC(6′)-Iak, and amikacin, isepamicin, kanamycin, and tobramycin were partially acetylated by AAC(6′)-Iak under the experimental conditions used. The TLC data for apramycin, gentamicin, and lividomycin were consistent with the MICs of the aminoglycosides for *E. coli* with pSTV28-*aac(6′)-Iak* and *E. coli* with the control vector (Table 1).

The substrate specificity of AAC(6′)-Iak was similar to that of AAC(6′)-Iz, although some kinetic parameters of AAC(6′)-Iak were different from those of AAC(6′)-Iz; i.e., the K_m for netilmicin and the k_{cat} for tobramycin of AAC(6′)-Iak were different from those of AAC(6′)-Iz (Table 2). The chemical structure of netilmicin is similar to that of sisomicin except for a residue at position 1 in 2-deoxystreptamine ring II (position R_2; ethylamino and amino groups, respectively). The ethylamino group at position R_2 in netilmicin, therefore, must be critical for the substrate affinity of AAC(6′)-Iz but not that of AAC(6′)-Iak. The chemical structure of tobramycin is similar to that of dibekacin, but tobramycin has a hydroxyl group at position 4′ in ring I (position R_1), whereas dibekacin does not, indicating that the hydroxyl group of tobramycin at position R_1 negatively affects the turnover rate (k_{cat}) of AAC(6′)-Iak but not that of AAC(6′)-Iz.

The structure of the genetic environment surrounding *aac(6′)-Iak* was similar to that of a region surrounding *aac(6′)-Iam* in *S. maltophilia* K279a, obtained from a patient in the United Kingdom (10) (Fig. 3). The genetic environment surrounding *aac(6′)-Iak* from nucleotides (nt) 23623 to 46040 had 92% identity to a genetic region in *S. maltophilia* K279a (accession no. AM743169) from nt 3660929 to 3683268 (10). The genetic environment surrounding *aac(6′)-Iak* in *S. maltophilia* IOMTU250 contained at least 5 housekeeping genes, including *purA*, *acnA*, *aroK*, *aroB*, and *thrA*, indicating that *aac(6′)-Iak* was located in the chromosomal genome.

All the *aac(6′)-Iz*, *aac(6′)-Iak*, and *aac(6′)-Iam* genes were detected in clinical isolates of *S. maltophilia* (10, 11, 17). These genes contributed to decreased susceptibility to 2-deoxystreptamine aminoglycoside antibiotics, such as neomycin, netilmicin, sisomicin, and tobramycin but not gentamicin (Table 1) (17). The deletion of *aac(6′)-Iz* in a clinical isolate of *S. maltophilia* resulted in the increased susceptibility of the isolate (17).

This study was approved by the Institutional Review Board of

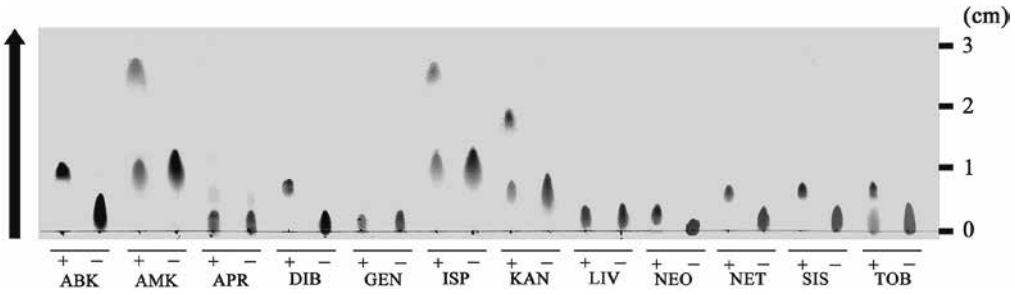

FIG 2 Analysis of acetylated aminoglycosides by thin-layer chromatography. AAC(6′)-Iak and various aminoglycosides were incubated in the presence (+) or absence (−) of acetyl coenzyme A. ABK, arbekacin; AMK, amikacin; APR, apramycin; DIB, dibekacin; GEN, gentamicin; ISP, isopamicin; KAN, kanamycin; LIV, lividomycin; NEO, neomycin; NET, netilmicin; SIS, sisomicin; TOB, tobramycin.

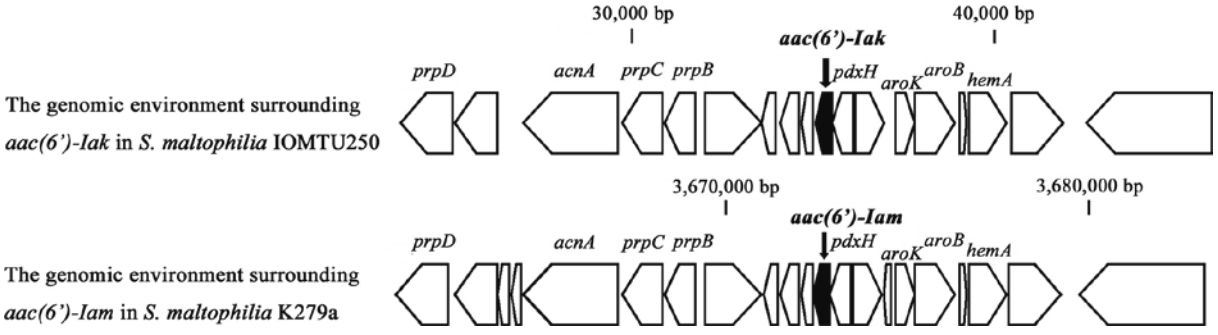

FIG 3 Genetic environments surrounding *aac(6′)-Iak* in *S. maltophilia* IOMTU250 and *aac(6′)-Iam* in *S. maltophilia* K279a.

the Institute of Medicine at Tribhuvan University (approval 6-11-E) and the Biosafety Committee of the Research Institute of the National Center for Global Health and Medicine (approval 25-M-038).

Nucleotide sequence accession number. The sequence for *aac(6′)-Iak* and its genetic environments (76,559 bp) was deposited in GenBank under accession number AB894482.

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Original article

Nosocomial Bacterial Infection and Antimicrobial Resistant Pattern in a Tertiary Care Hospital in Nepal

Sah MK[1], Mishra SK[2], Ohora H[3], Kirikae T[3], Sherchan JB[1], Rijal BP[2] and Pokhrel BM[2]

[1]Department of Microbiology, Kathmandu University and [1]Kantipur Dental College, Teaching Hospital & Research Center, Kathmandu, Nepal.

[2]Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal.

[3]National Center for Global Health and Medicine, Shinjuku, Tokyo, Japan.

Correspondence: Manoj Kumar Sah

E-mail: yourmanoj_sah@yahoo.com.

Abstract

Introduction: Nosocomial infection is a global problem with multi facet outcomes. At present, the emergence of resistance to antimicrobial agents is a global public health problem which is well pronounced in developing countries.

Methods: The aim of this study was to determine the prevalence of bacteria causing nosocomial infections and their antibiotics resistant pattern among the patients admitted at Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal. The study was conducted during a period of March 2011 to February 2012. Nine hundred clinical specimens which included urine, sputum, endotracheal aspirates, pus & blood were subjected for bacterial culture and their antibiotics sensitivity test at the Department of Microbiology with the use of standard method as described by American Society for Microbiology (ASM).

Results: Prevalence of bacteria causing nosocomial infection was 34.4% (n=310). Out of 310 specimens, urine 122 (39.30%), sputum 78(25.2%), pus 78(25.2%), endotracheal secreation 24 (7.7%) and blood 8(2.6%). Three hundred thirty three bacteria were isolated from three hundred ten specimens. The most common isolates were Escherichia coli followed by Acinetobacter species, Klebsiella pneumonia and Staphylococcus aureus. In-vitro antibiotic susceptibility tests revealed that the Gram-negatives bacilli were only sensitive to fluroquinolones, ceftrixone, cefepime carbapenem, polymyxin B and colistin sulphate while the Gram-positive cocci were sensitive to fluroquinolones, Ceftroxone, cefepime and vancomycin.

Conclusion: The findings suggested the need for constant monitoring of susceptibility of specific pathogens in different populations to commonly used anti-microbial agents to cope up this alarming situation in the hospital for the management of such patients and prevent the dissemination of such strains.

Key words: Nosocomial infections, Bacteria and Antibiotics

Introduction

Nosocomial infections, also called healthcare acquired infections or health care-associated infections, is defined by the Center for Disease Control (CDC) as a localized or systemic condition that results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) and that was not present or incubating at the time of admission to the hospital. For most bacterial nosocomial infections usually become evident after 48 hours (i.e., the typical incubation

period) or more after admission. However, because the incubation period varies with the type of pathogen and to some extent with the patient's underlying condition, each infection must be assessed individually for evidence that links it to the hospitalization [1].

Nosocomial infection is a problem throughout the world both in developed and developing countries. The changing pattern of the bacterial isolates causing nosocomial infection has been observed in different time period. The impact of nosocomial infection on public health is a subject of increasing concern, due to the increasing numbers of hospitalized patients in crowded facilities, many of whom have impaired immunity, the emergence of new micro-organisms, and the increase in antibiotic resistance. In many countries, strict guidelines and policies for control, prevention, and management of nosocomial infections are implemented but even then hospital infections do occur in one form or another. In Nepal, there is a lack of education in this field but other social, ethical and economic factors also need to be considered in the control of nosocomial infections.

Over 1.4 million people worldwide suffer from infectious complications acquired in hospital. The highest frequencies of nosocomial infections were reported from hospitals in the Eastern Mediterranean and South-East Asia Regions (11.8 and 10.0%, respectively), with a prevalence of 7.7 and 9.0%, respectively in the European and Western Pacific Regions. Twenty five to 50% of nosocomial infections are due to the combined effect of the patients own flora and invasive devices. Most infections acquired in hospital today are caused by microorganisms which are common in the general population, in whom they cause no or milder disease than among hospital patients (*Staphylococcus aureus*, coagulase-negative *Staphylococci*, *Enterococci*, Enterobacteriaceae) [2].

Nosocomial infections are also important public health problems in developing countries, as well as in developed countries. The socioeconomic impact, i.e. prolongation of hospitalization, mortality, and cost, of these infections adversely affects patients and nations economic well-being. They are important for both patient and public health problem in developing countries, as well as in some developed countries [3, 4]. Nosocomial infections may result in an excess length of stay in hospital for up to 10 days and an increase in the costs of hospitalization [5, 6]. Nosocomial infections pose a critical threat to patients, especially in the high-risk departments, such as the Intensive Care Unit (ICU) [7, 8]. Risk factors for the development of nosocomial infections in the Surgical Intensive Care Unit (SICU) setting include poor nutritional status, exposure to multiple antibiotics, indwelling central venous catheters; mechanical ventilation and length of ICU stay [9]. Over the past several decades, the frequency of antimicrobial resistance and its association with serious infectious diseases have increased at alarming rates. The increasing resistance rate among nosocomial pathogens is a commonly encounter problem [10, 11].

It is estimated that in developed countries 5–10% patients get one of these infections during hospitalizations, whereas in developing countries rates are higher up to (25%) [12]. An international study covering 47 hospitals in 14 countries (Europe, Eastern Mediterranean, Southeastern Asia and Western Pacific Region) over the period from 1983 to 1985 showed that an average prevalence rate was 8.7%, ranging from 3 to (21%) [13].

Today, antibiotic remain the front line therapy for conquering bacterial infections. However, treatment with these drugs is to be acknowledges as a two edged sword. As antimicrobial agents have been misused and overused, bacteria have fought back with a selection process by which certain strains are now no longer susceptible to one or more agents. As a result, bacteria that once seemed to be losing the battle for survival have re-emerged to create therapeutic dilemmas with resulting increased risks of treatment failure and disease complications. As the incidence of antimicrobial resistance rises, so do costs associated with its consequences. The worldwide emergence of multidrug resistance (MDR) among Gram-negative and Gram-positive bacteria has resulted in a great threat to conquer against the microbes.

During invasive procedures pathogens that are present on medical personnel hands or in the instruments or that are acquired by the patient in the skin, respiratory tract, genitourinary tract, gets entry into the already weakened patients. These medical procedures bypass natural protective barrier against the entry of pathogens and provide an easy route for infection. Patients already colonized with hospital strains on admission are instantly put at a greater risk when they undergo such invasive procedure leading to nosocomial infections.

Method

A Prospective study was conducted from March 2011-February 2012 at intensive care unit, medical wards, orthopedic ward, neurological ward, surgical ward, surgical ICU and Department of Microbiology, TUTH. A total of 900 specimens which included urine, sputum, pus, endotracheal secretions and blood were collected from patients admitted at TUTH. All the specimens were collected, culture, identification tests were done by according to the standard protocol by the ASM and

analyzed accordingly [14]. The antibiotic sensitivity tests of the pathogens isolated from the clinical specimen against different antibiotics were done using Mueller Hinton agar by the standard disk diffusion technique of Kirby- Bauer method as recommended by CLSI [15]. This study was approved by Institutional Review Board of Institute of Medicine. Data were analyzed by using SPSS version 17.0.

A detailed clinical examination and review of systems most likely reveal the involved organs or systems. Investigation should be focused on these abnormal areas such as; bloodstream, UTI, pneumonia and surgical-site infection. Laboratory test for nosocomial infection can be performed by taking specimens from the sites of the infection [16]. Laboratory analyses aim to identify the responsible infectious agent, evaluation of its susceptibility to anti-infectious treatments, typing of bacterial strains etc. The identification of common nosocomial infection sites and simplified criteria for each infection. (Table: 1)

Table 1 Provides common nosocomial infection sites and Simplified criteria for each infection[2].

Type of nosocomial infections	Simplified criteria
Surgical site infection	Any purulent discharge, abscess, or spreading cellulitis at the surgical site during the month after the operation
Urinary infection	Positive urine culture (1 or 2 species) with at least 10^5 bacteria/ml, with or without clinical symptoms
Respiratory infection	Respiratory symptoms with at least two of the following signs appearing during hospitalization: — Cough — Purulent sputum — New infiltrate on chest radiograph consistent with infection
Vascular catheter infection	Inflammation, lymphangitis or purulent discharge at the insertion site of the catheter
Septicemia	Fever or rigours and at least one positive blood culture

Result

Nine hundred patients admitted between March 2011 to February 2012 at Tribhuvan University Teaching Hospital were studied for prevalence of nosocomial infections. On admission, they were carefully examined clinically as well as microbiologically to exclude community-acquired infections and to determine any underlying risk factors. Out of nine hundred specimens 34.4% (n= 310) were found to be associated with nosocomial infection. (Table 2 and Figure 1)

Table 2 Prevalence of nosocomial infection

Total no. of specimens	900
Specimens those associated with nosocomial infection	310

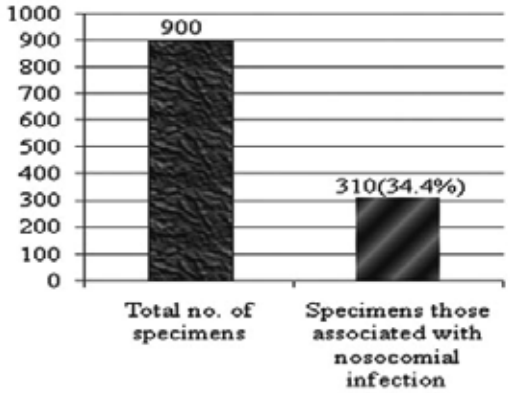

Figure 1. Prevalence of nosocomial infection

Distribution of specimens associated with nosocomial infections

Out of 310 specimens, urine 122 (39.30%), sputum 78(25.2%), pus 78(25.2%), endotracheal secreation 24 (7.7%) and blood 8(2.6%). (**TABLE 3)**

Table 3 Distribution of specimens associated with nosocomial infections

Specimens	Number	Percent
Urine	122	39.3
Sputum	78	25.2
Pus	78	25.2
ET secretion	24	7.7
Blood	8	2.6
Total	310	100

Three hundred thirty three bacteria were isolated from three hundred ten specimens. Among the 122 urinary bacterial isolates, Escherichia coli was found to be the most predominant (41.8%) followed by Enterococcus faecalis (14.8%), Acinetobacter spp. (15.6%), Klebsiella pneumoniae (9%), Pseudomonas aeruginosa (9%), Staphylococcus aureus (9%) and Citrobacter freundii (0.8). In case of sputum specimens (n=79), K. pneumoniae was found to be most predominant bacteria (25.3%) which was followed by Acinetobacter spp. (3%), E. coli (16.5%), P. aeruginosa (10.1%), S. aureus (19%). Whereas (n=95) bacteria were isolated from pus specimens, E. coli (31.6%) was most common pathogen which is followed by Acinetobacter spp. (20.0%), K. pneumoniae (13.7%), P.aeruginosa (11.6%), C. freundii (4.2%), M. morgannii (2.1%) S. aureus (15.8%) and E. faecalis (1.1%).Among the 29 endotracheal bacterial isolates, Acinetobacter spp was found to be more predominant (44.8%) which was followed by P. aeruginosa (24.1%), K. pneumoniae (20.7%) and E.coli (10.3%). Moreover, eight bacteria were isolates from blood in which Acinetobacter spp. was found to be more predominant (50%) which was followed by C. freundii (12.5%), E. coli (12.5%) and S. aureus (25%). (Table 4)

Table 4 Distribution of Bacteria associated with Nosocomial Infection

Sites of Nosocomial Infection	Bacterial Isolates	Number	Percent
UTI (n=122)	Escherichia coli	51	41.8
	Acinetobacter spp.	19	15.6
	Klebsiella pneumoniae	11	9
	Pseudomonas aeruginosa	11	9
	Citrobacter freundii	1	0.8
	Enterococcus spp.	18	14.8
	Staphylococcus aureus	11	9
LRTI (n=79)	K. pneumoniae	20	25.3
	Acinetobacter spp.	20	25.3
	E.coli	13	16.5
	P.aeruginosa	8	10.1
	C.freundii	3	3.8
	S.aureus	15	19
SSI (n=95)	E.coli	30	31.6
	Acinetobacter spp.	19	20.0
	K.pneumoniae	13	13.7
	P.aeruginosa	11	11.6
	C.freundii	4	4.2
	M.morgannii	2	2.1
	S.aureus	15	15.8
	E.faecalis	1	1.1
VAP (n=29)	Acinetobacter spp	13	44.8
	P.aeruginosa	7	24.1
	K.pneumoniae	6	20.7
	E.coli	3	10.3
BSI (n=8)	Acinetobacter spp.	4	50
	C.freundii	1	12.5
	E.coli	1	12.5
	S.aureus	2	12.5

Table 5 Distribution of bacteria associalted with nosocomial infection

Antibiotics	E. coli (n=51)	A. spp. (n=19)	K. pneumoniae (n=11)	P. aeruginosa (n=11)	E. spp. (n=18)	S. aureus (n=11)
Amoxycillin	98	100	100	-	100	100
Ciprofloxacin	96	100	100	100	77.8	81.8
Co-trimoxazole	86.3	40	100	-	-	81.8
Nitrofurantoin	39.2	94.7	81.8	-	33.3	18.2
Norfloxacin	86.3	100	100	100	83.3	81.8
Cephalexin	94.2	100	100	-	-	90.9
Ceftriaxone	86.3	47.4	100	54.5	-	-
Cefotaxime	86.3	52.6	100	54.5	-	-
Ceftazidime	86.3	47.4	100	54.5	-	-
Cefepime	68.6	36.8	90.9	36.4	-	-
Gentamycin	64.7	100	90.9	90.9	-	63.6
Amikacin	35.3	89.5	63.6	81.8	-	45.5
Ampicillin-Sulbactam	92.2	68.4	100	-	-	-
Cefoparazone -Sulbactam	17.6	36.8	54.5	45.5	-	-
Piperacillin	82.4	57.9	81.8	45.5	-	-
Piperacillin-Tazobactam	29.5	26.3	54.5	45.5	-	-
Imipenem	5.9	31.6	27.3	36.4	-	-
Meropenem	5.9	26.3	27.3	36.4	-	-
Polymyxin B	0	0	0	0	-	-
Colistin sulphate	0	0	0	0	-	-
Erythromycin	-	-	-	-	94.5	90.9
Vancomycin	-	-	-	-	0	0
Cloxacillin	-	-	-	-	-	54.5
Clindamycin	-	-	-	-	-	63.6

Incidence of antibiotics resistant with E. coli to ciprofloxacin (96%), cephalosporin(86.3%), gentamycin (64.7%), nitrofurantoin (39.2%), Acinetobacter spp. to cephalosporin (100%), ciprofloxacin (100%), Klebsiella pneumoniae to ceftriaxone (100%), amikacin 89.5%), P. aeruginosa to ceftazidime (54.5%), piperacillin (45.5%) and piperacillin-tazobactam (45.5%), Enterococcus spp. to ciprofloxacin (77.8%), and S. aureus to amoxycillin (100%), ciprofloxacin (81.8%), cloxacillin (54.5%) and cefalexin (90.9%) in urinary isolates.(Table 5)

Table 6 Antimicrobial Resistant Pattern of Urinary Isolates Presented in Percentage

Antibiotics	K. pneumoniae (n=20)	A. spp. (n=20)	E. coli (n=13)	P. aeruginosa (n=8)	S. aureus (n=15)
Amoxycillin	100	100	100	-	100
Ciprofloxacin	85	95	92.3	62.5	86.7
Ofloxacin	85	95	84.6	62.5	86.7
Co-trimoxazole	85	100	92.3	-	73.3
Cephalexin	100	100	92.3	-	93.3
Ceftriaxone	95	100	92.3	62.5	93.3
Cefotaxime	95	100	92.3	62.5	93.3
Ceftazidime	95	100	92.3	75	-
Cefepime	85	95	77	50	93.3
Gentamycin	90	95	84.6	75	80
Amikacin	65	95	53.8	62.5	73.3
Ampicillin-Sulbactam	-	100	92.3	-	-
Cefoparazone -Sulbactam	45	80	38.5	12.5	-
Piperacillin	90	95	92.3	87.5	-
Piperacillin -Tazobactam	55	95	69.2	25	-
Imipenem	15	95	23	12.5	-
Meropenem	15	95	23	12.5	-
Polymyxin B	0	0	0	0	-
Colistin sulphate	0	0	0	0	-
Erythromycin	-	-	-	-	100
Vancomycin	-	-	-	-	0
Cloxacillin	-	-	-	-	66.7
Clindamycin	-	-	-	-	66.7

Bacteria isolated from sputum specimens showed antibiotics resistant with K. pneumoniae to ceftriaxone (95%), amikacin (65%), Acinetobacter spp. to cephalosporin (100%), ofloxacin (95%), E. coli to cephalosporin (92.3%), carbapenem (23%), P. aeruginosa to ceftazidime(75%), piperacillin (87.5%) and piperacillin-tazobactam (25%) and S. aureus to amoxycillin (100%), ofloxacin (86.7%), cloxacillin (66.7%) and cefalexin (93.3%).(Table 6)

Table 7 Antimicrobial Resistant Pattern of Sputum Isolates Presented in Percentage

Antibiotics	*E. coli* (n=30)	A. spp. (n=19)	*K. pneumoniae* (n=13)	*P. aeruginosa* (n=11)	*S. aureus* (n=15)
Amoxycillin	100	100	100	-	100
Ciprofloxacin	96.7	100	100	100	86.7
Ofloxacin	96.7	100	100	100	86.7
Co-trimoxazole	76.7	80	100	-	80
Cephalexin	96.7	94.7	100	-	93.3
Ceftriaxone	83.3	89.5	100	81.8	67.7
Cefotaxime	83.3	89.5	100	81.8	67.7
Ceftazidime	83.3	89.5	100	81.8	-
Cefepime	76.7	80	92.3	63.6	67.7
Gentamycin	73.3	94.7	100	90.1	73.3
Amikacin	40	84.2	92.3	90.1	46.7
Ampicillin-Sulbactam	90	68.4	100	-	-
Cefoparazone -Sulbactam	23.3	40	92.3	45.5	-
Piperacillin	93.3	84.2	100	63.6	-
Piperacillin-Tazobactam	36.7	57.9	77	63.6	-
Imipenem	6.7	63.2	38.5	54.5	-
Meropenem	6.7	63.2	38.5	54.5	-
Polymyxin B	0	0	0	0	-
Colistin sulphate	0	0	0	0	
Erythromycin	-	-	-	-	93.3
Vancomycin	-	-	-	-	0
Cloxacillin	-	-	-	-	67.7
Clindamycin	-	-	-	-	73.3

The rate of antibiotics resistant with E. coli to ceftriaxone (83.3%), carbapenem (6.7%), Acinetobacter spp. to cephalosporin (89.5%), ofloxacin (100%), K.pneumoniae to ceftriaxone (95%), amikacin (92.3%), P. aeruginosa to ceftazidime (81.8%), piperacillin (63.6%) and piperacillin-tazobactam (63.6%) and S. aureus to amoxycillin (100%), ofloxacin (86.7%), cloxacillin (67.7%) and cefalexin (93.3%) in pus isolates.(Table 7)

Table 8 Antimicrobial Resistant Pattern of Pus Isolates Presented in Percentage

Antibiotics	Acinetobacter spp. (n=13)	K. pneumoniae (n=6)	P. aeruginosa (n=7)
Amoxycillin	100	100	-
Ciprofloxacin	100	100	100
Co-trimoxazole	100	16.7	-
Ofloxacin	100	100	71.4
Cephalexin	100	100	
Ceftriaxone	100	100	100
Cefotaxime	100	100	100
Ceftazidime	100	100	100
Cefepime	100	16.7	71.4
Gentamycin	100	100	85.7
Amikacin	100	16.7	85.7
Ampicillin-Sulbactam	100	16.7	-
Cefoparazone -Sulbactam	92.2	66.7	28.6
Piperacillin	100	100	42.9
Piperacillin-Tazobactam	100	100	14.3
Imipenem	92.2	0	28.6
Meropenem	92.2	0	28.6
Polymyxin B	0	0	0
Colistin sulphate	0	0	0

Bacteria isolated from endotracheal screation revealed antibiotics resistant with Acinetobacter spp. showed 100% resistant to most commonly prescribed antibiotics except carbapenem, cefoparazone-salbactam (92.2%) respectively, K. pneumoniae to amikacin (16.7%), cefoparazone-salbactam (67.7%), P. aeruginosa to ceftazidime (100%), piperacillin (42.9%) and piperacillin-tazobactam (14.3%) (Table 8)

The antibiogram of blood isolated, for *Acinetobacter* spp. the antibiotic effect was very poor. They showed 100% sensitive to Polymyxin B and colistin sulphate followed by cefoperazone-sulbactam (75%), Piperacillin-tazobactam (75%), imipenem (75%). *E.coli* were found to be resistant to amoxycillin, aminoglycosides and second generation of cephalosporin, and sensitive to polymyxin B and colistin sulphate, imipenem and meropenem and others. For *S.aureus*, amoxycillin had no effect. Vancomycin were found to be most effective antibiotic (100%) which was followed by amkacin and cloxacillin (each 50%).

Discussion

The study was aimed to find out the current prevalence and trend of the bacteria causing nosocomial infections and the efficacy of drugs being used against them. The overall prevalence of bacteria causing nosocomial infection is (34.4%), which is higher than the similar studies in the other hospitals from different countries, which were

(13%-17.8%) [17-22]. This increase in the prevalence of nosocomial infections in this hospital may be attributed to less attention being paid to well-established processes for decontamination and cleaning of soiled instruments and other items, followed by sterilization and high-level disinfection processes and improving safety in operating rooms and other high-risk areas where the most serious and frequent injuries and exposures to infectious agents occur.

In this study, the most common nosocomial infection was found to be UTI (39.30%) followed by LRTI (25.20%), SSI (25.20%), VAP (7.7%) and BSI (2.6%). Our results are concurrent with the multicentic study in Greece showed that UTI was (22.4–38.2%), LRTI (21.1–32.6%), SSI (14.6–22.7%) and BSI (9–13.2%) [23].

The bacteria isolated in our study from patients who were suffered from nosocomial urinary tract infections included E. coli (41.8%) followed by Acinetobacter spp. (15.6%), Enterococcus spp. (14.8%) and S. aureus (9%).These results were supported by Neto et al. (2003) study which was done among 188 patients with positive urine culture in Brasileira and found that the most common pathogens causing nosocomial urinary tract infections were E-coli (26%), Klebsiella spp. (15%), P. aeruginosa (15%) and Enterococcus spp. (11%)[24].

In this study K. pneumoniae was found to be most predominant bacteria (25.3%) causing nosocomial LRTI followed by Acinetobacter spp. (25.3%), E. coli (16.5%) and P. aeruginosa (10.1%). In a study by Singh et al, most frequent isolates causing LRTIs were Klebsiella spp. (24.48%), followed by Proteus (18.33%) and E. coli (12.24%) which concurrent with our study [25]. This shows that the prevalence of K. pneumoniae has increased in 2012 as compared to 2010 at TUTH. A study done by Mishra et al showed the growth of 18.95% of K. pneumoniae in lower respiratory tract infection.

In the surgical site infection (SSI), E. coli (31.6%) were found to be most predominant followed by Acinetobacter spp. (20%), K. pneumonia (13.7%), P. aeruginosa (11.6%), C. freundii (4.2%), M. morgannii (2.1%) and S. aureus (15.8%), Enterococcus spp.(1.1%). Regarding the growth pattern, single bacterial growth was found in 10.5% of the cases while 79.5% were multiple bacterial growths (2 or more than 2). This could be because of the profound influence of endogenous contamination from the bowel and hollow muscular organs of patients.

In case of ventilator associated pneumonia (VAP), Acinetobacter spp. was found to be more predominant (44.8%) followed by P. aeruginosa (24.1%), K. pneumonia (20.7), E. coli (10.3%). A study conducted in Nepal by Ranjit S, Bhattarai B, Acinetobacter spp.was most common bacteria causing VAP[26]. Gram negative bacteria, P. aeruginosa and Acinetobacter baumanii are commonly associated with late onset VAP [27].

In case of nosocomial blood stream infection (BSI), Acinetobacter spp. was found to be more predominant (50%) followed by C. freundii (12.5%), E. coli (12.5%) and S. aureus (25%).

Currently many microorganisms have become resistant to different antimicrobial agents and in some cases to nearly all agents. Resistance to antimicrobial agents is a problem in health care facilities, but in hospitals, transmission of bacteria is amplified because of the highly susceptible population (WHO, 2002). The antibiotic resistant of our study confirmed the alarming percentage of resistance exhibited by pathogens to the common antibiotics in use.

However, the present study showed a high prevalence of resistance to the commonly prescribed antimicrobial agents. This may be because of the intense use of antimicrobial agent in the hospital, easy availability and indiscriminate use of these drugs outside the hospitals, and many antibiotics are available over the counter for self-medication. These problems, coupled with the increase chance of cross infection among inpatients, are known to account for circulating resistance strains.

The emergence of Gram-negative bacterial species with acquired resistance to various broad spectrum β-lactams and other classes of antimicrobials is becoming a worldwide clinical problem. This may be due to exposure of hospitalized patients to different broad and extended spectrum drugs beside multiresistant isolates are disseminated widely in the hospital setting due to different iatrogenic mechanism and these patients may not be immunocompetent.

This study provides insights into the problem of resistance in bacterial pathogens in TUTH. Our results demonstrated that, in general, isolates have high rates of resistance to antibiotics commonly used in developing countries. We also found a high rate of resistance to amoxicillin, first, second and third generation cephalosporins, fluroqunolones, aminoglycosides and co-trimoxazole. Therefore, cheap antibiotics such as amoxicilline, ciprofloxacin, gentamycin, cephalexin and co-trimoxazole are now of limited benefit in the treatment of infections in TUTH.

The high level of ciprofloxacin resistance among E. coli, and more generally Enterobacteriaceae, rules out the use of ciprofloxin as empirical treatment when invasive infections due to these pathogens are suspected. The rate of resistance to third-generation cephalosporins is also worrisome.

The high prevalence of Acinetobacter spp. in UTI 15.5%

(n=19), LRTI 25.3% (n=20), SSI 20% (n=19) and in P. aeruginosa UTI 9% (n=11), LRTI 10.1% (n=8), SSI 11.6% (n=11) may have been exacerbated by failure of infection control in the hospitals. The overall rate of antibiotic resistance in Acinetobacter spp. was higher than that in P. aeruginosa, this observation contrasts with previous results founds in South Africa[28]. Resistance to carbapenem (imipenem) in Acinetobacter spp. was (31.6-95) %, but (12.5-54.5) % in P. aeruginosa. This high rate of resistance to carbapenem in Acinetobacter spp. in our study is striking given that this antibiotic is frequently prescribed in TUTH, Nepal. This result may be due to the clonal spread of a multi-resistant strain of A. baumanii.

The indication of antibiotic therapy for nosocomial UTIs in acute care settings is a controversial issue. Nonetheless, the treatment of symptomatic UTIs is virtually universal. Yet routine therapy increases not only drug costs but also adverse drug reactions and the emergence of antibiotic–resistant microorganisms. The increasing antimicrobial resistance among the bacteria causing nosocomial urinary tract infections makes therapy of this type of infections difficult and leads to more use of extensive broad-spectrum drugs.

Carbapenem resistance in Acinetobacter was widespread. Carbapenems therefore can no longer be relied on as empiric therapy for these organisms, leading to an increase in use of alternatives such as polymyxin B and colistin. We found that 100% were susceptible to polymyxin B and colistin sulphate.

Carbapenems have potent activity against multidrug resistant Acinetobacter isolates. Acinetobacter spp. may develop resistance to carbapenem through various mechanisms including class B and D carbapenemase production, decreased permeability, altered penicillin binding proteins and rarely over expression of efflux pumps [29, 30]. The resistance of Acinetobacter spp. towards the carbapenems is much higher in this study as compared to different studies in Indian hospitals at All India Institute of Medical Sciences (AIIMS) (34.7% for meropenem and 27.2% for imipenem)[31].

Conclusion

It is quite alarming that prevalence of bacteria causing nosocomial infection was 34.4% in TUTH. This study showed that Gram-negative bacilli were the predominant isolates. Polymyxin B, colistin sulphate, imipenem, meropenem and nitrofurantoin were relatively effective drugs for Gram-negative bacilli where as vancomycin was relatively effective drugs for Gram-positive cocci. However, all the bacteria isolated from nosocomial infection were 100% resistance to Ampicillin. Empirical treatment to nosocomial infections provoke drug resistance, therefore treatment should be based on the result of culture and sensitivity. This study concludes that if one could not wait the culture results in nosocomial infection amoxicillin, cloxacillin, ciproflocacin, gentamycine are quite ineffective to treat these infections.

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Clinical epidemiology and molecular analysis of Extended-spectrum β-Lactamase (ESBL)-producing *Escherichia coli* in Nepal: Characteristics of *E. coli* Sequence Type ST131 and ST648

Jatan. B. Sherchan, MD,[1] Kayoko Hayakawa, MD, PhD,[2#] Tohru Miyoshi-Akiyama, PhD,[3] Norio. Ohmagari, MD, MSc,[2] Teruo Kirikae, MD, PhD,[3] Maki Nagamatsu, MT[2,3] Masayoshi Tojo, MD, PhD,[2,3] Hiroshi Ohara, MD, PhD [4] Jeevan B Sherchand, PhD[5] Sarmila Tandukar, MSc[5]

Department of Clinical Microbiology, Kathmandu University School of Medical Sciences, Dhulikhel, Nepal[1]; Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan[2]; Department of Infectious Diseases, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan[3]; Department of International Medical Cooperation, National Center for Global Health and Medicine, Tokyo, Japan;[4] Public Health Research Laboratory, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal[5]

Key words: ESBL; Extended-spectrum β-lactamase; CTX-M; *E. coli*; Antimicrobial resistance, whole genome sequence

Abstract

Background: Recently, CTX-M type extended-spectrum beta-lactamase-producing *E.coli* (ESBL-*E. coli*) have emerged worldwide. Especially, *E. coli* O antigen type 25 (O25) and sequence type (ST) 131, often associated with the CTX-M-15 ESBL, has been increasingly reported globally. However, epidemiology reports on ESBL-*E.coli* in Asia are limited.

Methods: Patients who had clinical isolates of ESBL- *E. coli* in Tribhuvan university teaching hospital in Kathmandu, Nepal were included. To analyze MLST, phylotypes, virulence genotypes, O25b-ST131 clone, and distribution of acquired drug resistance genes, whole genome sequence of the isolates were conducted.

Results: During the study period, total of 105 patients with ESBL-*E. coli* isolation were identified, and majority (90%) of the ESBL-*E. coli* isolates were CTX-M-15 positive. The most dominant ST was ST131 (n=54 [51.4%]), followed by 15 (14.3%) cases of ST648. Among the 54 ST131 isolates, all 54 (100%) were identified as O25b-ST131 clone as well as the H30-Rx subclone. Analyses comparing 3 groups (ST131, ST648, non ST131/648) were conducted. Higher proportion of ST648 isolates had resistance to non-beta lactam antibiotics than ST131 or non-ST131/648, and possessed drug resistant genes more frequently than ST131 or non-ST131/648. ST131 had virulence genes most frequently, followed by ST 648. Clinical characteristics were similar among groups.

More than 38% of ESBL-*E. coli* were isolated from outpatient clinic, and pregnant patients consisted 24% of ESBL-*E.coli* cases.

Conclusions: We identified that the highly resistant status of ESBL-*E coli* to multiple classes of antibiotics in Nepal are mainly driven by CTX-M-producing ST131 and ST648. Their immense prevalence in communities is of great concern.

Antimicrobial Agents and Chemotherapy

Assessment of health systems in relation to interface between malaria control programs and health system strengthening: Comparative study between Nepal and Viet Nam

Hiroshi Ohara, Yasuyo Matsumoto, Takanori Hirayama, Hidechika Akashi, Jeevan B Sherchand, Vu Huy Nam, Jatan B Sherchan

Bureau of International Medical Cooperation, National Center for Global Health and Medicine, Japan

Institute of Medicine, Tribhuvan UniversityFaculty of Medicine, Kathmandu University

National Institute of Malariology, Parasitology and Entomology

Abstract

Malaria control has been a major health issue with high priority in endemic countries and various efforts have been made supported by foreign assistant partners. In order to implement efficient and sustainable control, integration of the control program into general health system or effective interactions between them is one of the important strategies.

Studies were conducted in Vietnam and Nepal. Information obtained from the document reviews, interviews, and field surveys were analyzed from the viewpoint of interface between malaria control program and the health system in accordance with the 6 building blocks of a health system with special emphasis on good practices and challenges in implementing malaria control program.

Among good practices, strong government commitment on the control programs strengthening of facilities and capacity of health workers at primary level, utilization of health volunteers, setting up mobile teams, intensified education for residents were noteworthy. Key challenges were mainly existing in remote areas. Introduction of malaria due to population movement and the emergence of new endemic areas have become growing issues. Strengthening of the vertical health program appeared to have some impact on the general health system, particularly at the primary level. However, it can be said that dissociation between vertical control program and horizontal general health system also exists.

It is crucial to implement effective and equitable malaria control program addressing existing challenges and to create sustainable health systems. These tackling will lead to further strengthening of the health system there and eventually effective implementation of various health programs.

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Contact details for further information

Hiroshi OHARA, M.D., Ph.D.
Bureau of International Health Cooperation, Japan
National Center for Global Health and Medicine, Japan
Email: oharah@it.ncgm.go.jp

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IOM-NCGM Research Collaboration Office

National Center for Global Health and Medicine, Japan
Bureau of International Health Cooperation, Japan
1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655
Tel: +81-3-3202-7181 / Fax: +81-3-3205-6780
info@it.ncgm.go.jp

Research Collaboration Office

Institute of Medicine, Tribhuvan University, Kathmandu, Nepal

National Center for Global Health and Medicine, Tokyo Japan