Annual Report

2013

IOM-NCGM
Research Collaboration Office

March 2014
Kathmandu, Nepal
Tokyo, Japan

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Preface

The Medical Education Project 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 and its attached teaching hospital (TUTH). I was dispatched as a team leader for the last two years of the project (1994-1996), and worked towards the success of the project in close cooperation with the staff of IOM. 17 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.

Good relationship was built between NCGM and IOM during the project period and it has been maintained until today. This is one of the valuable outcomes and legacies of the project. After the conclusion of Memorandum of Understanding (MOU) in January 2013 between National Center for Global Health and Medicine (NCGM) and IOM, a unique cooperation focusing mainly on research and human resource development has been created and developed. I am very grateful to have a good relationship like this.

Collaborations initiated based on the MOU are on track, and a number of results have begun to appear. At this time, we could summarize the outline report of our new collaboration. We will do our best to implement useful activities for both countries' benefit through the continued collaborative activities.

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 NCGM and IOM will be further strengthened.

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

Senior Advisor

Bureau of International Medical Cooperation

National Center for Global Health and Medicine

Tokyo, Japan

त्रिभुवन विश्वविद्यालय
चिकित्सा शास्त्र अध्ययन संस्थान
डीनको कार्यालय, महाराजगंज
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Preface

It is our great pleasure to know that Dr. Hiroshi Ohara, National Center for Global Health and Medicine (NCGM) has written an annual report of our collaboration between NCGM and Tribhuvan University, Institute of Medicine. We would like to express our sincere thanks to NCGM for helping to promote research on infection control. As a result, Dr. Hiroshi Ohara, Dr. Teruo Kirikal and other Japanese scientists and Nepalese Scientists have opportunity to express their academic potentiality in the respective field. During our collaboration between IOM and NCGM, we have been able to discover new strain of MDR organism in Nepal, Such as NDM-1, NDM-8 and fact finding survery of nosocomial infection control situation in Nepal. We assured, that this finding will contribute the way for possible preventive measures. During the study period, one of our achievements was to attend international conference held in Okayama and Nagasaki in the year 2012 and 2013.

Inaddition, four of our scholars have received academic support to visit NCGM, Japan to learn advance techniques for their study.

We would like express our gratitude to NCGM, specially to Dr. Ohara, Dr. Kirikae and other members of NCGM.

We look forward to continue this collaboration between two institutions in the future endevour.

Prof. Dr. Bharat Mani Pokhrel
Assistant Dean

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

Abbreviations

COPD	Chronic Obstructive Pulmonary Disease
CT	Computed Tomography
DM	Diabetes Mellitus
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
MRI	Magnetic Resonance Imaging
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.

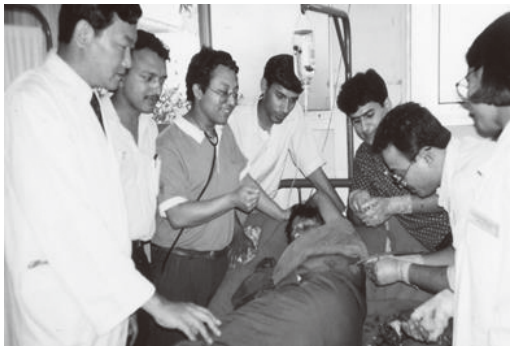
2. Background

1) Medical Education Project

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 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. 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.

Tribuvan University Institute of Medicine (IOM)

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. 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.



Technical Cooperation in Medical Education Project (1995)

Symposium on Infection Control (2009)

2) After Completion of the Technical Cooperation Project

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, 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. Due to continued unstable political conditions in Nepal, cooperative relations 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. In 2013, the “Joint Conference on Infectious Diseases with Growing Concern in Recent Years in Nepal” was held at IOM.

3. Conclusion of MOU and the Establishment of NCGM-IOM Research Collaboration Office

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 (left) and IOM (right) (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, although the office is being prepared, activities utilizing grants from the International Health Cooperation Research (24-5, 25-5, 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

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.

5. 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.

Visiting Kathmandu University
School of Medical Sciences to discuss
research collaboration on multi-drug resistant bacteria

II. 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 2013.

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 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

- 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

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

- Recently in Nepal, although there has been increased awareness regarding nosocomial infection control, implementation of control measures is still limited. 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 2013 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 2013, NCGM-IOM Research Collaboration Office

Collaboration with Nursing Director of TUTH
(right) to improve nosocomial infection control

2. Outline of each research

Table 1 shows overview of the collaborative researches between NCGM and IOM. Progress status of each research (No. 1-6) is described on pages 13-22. We have already obtained some results, and these

results were published at medical conferences and on scientific papers as shown on pages 49-91 (related papers in 2011 and 2012 are also included in addition to the papers in 2013). 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 (Cf. Pages 15-16, 71-73, 81-83)

Table 1 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	Oral presentation 1. 53th Annual Meeting of the Japanese Society of Tropical Medicine, October 2012, Obihiro, Japan 2. 54th Annual Meeting of the Japanese Society of Tropical Medicine, October 2013, Ngasaki, Japan Report (submitted to WHO) 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 primary 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. In Nepal, the general health system, which was fragile in the past, was strengthened and utilized in greater part in the malaria control program. During the period of political instability (1996-2006), health systems were affected, but the influence on malaria control was minimal compared with other disease control programs due to the high	

	governmental priority placed on malaria control and continuous support of the international community. 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. We made a further study in 4 districts in Terai areas 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. This implies that rich people were more likely to own and use bednets than their poor counterparts. 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	1. Int J Antimicrob Agens. 2013, 42(4):372-374 2. Antimicrob Agents Chemother. 2013, 57(5):2394-2396 Oral presentation 1. 42th Annual Meeting of the Society for Bacterial Drug Resistance, October 2013, Shizuoka, Japan

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 and his colleagues obtained 308 Gram-negative isolates by November 2013, including 91 (29.5%) *Acinetobactor baumannii*, 80 (26.0%) *Escherichia coli*, 53 (17.2%) *Klebsiella pneumoniae*, 19 (6.2%) *Pseudomonas aeruginosa*, and 9 (2.9%) *Stenotrophomonas maltophilia* isolates. Of 308 isolates, 194 isolates were highly resistant to all aminoglycosides tested, including amikacin, arbekacin and gentamicin, with MICs of more than 512 mg/L. These isolates had 16S rRNA methylase genes, including *armA*, *rmtB*, *rmtC*, *rmtF*.

Of the 80 *E. coli* isolates, we detected a novel NDM-type metallo-β-lactamase variant, NDM-8, in 5 isolates. The amino acid sequence of 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. The MICs of cefmetazole, cefoselis, cefpirome, doripenem, imipemen, panipenem, and moxalactam were one dilution higher for *E. coli* expressing NDM-8 than NDM-1. In contrast, those of ceftriaxone and meropenem were one dilution lower for NDM-8 than NDM-1. NDM-8 showed enzymatic activities against β-lactams similar to those of NDM-1.

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. Fourteen isolates belonged to clonal complex (CC) 14 and 5 to CC11. Thirteen isolates belonged to multilocus sequence type (MLST) ST15, 3 isolates to ST340, and 1 isolate each to types ST11, ST14, ST29, ST43, ST378, ST395, ST437, ST1231 and ST1232. Pulsed-field gel electrophoresis (PFGE) pattern analysis showed 2 clusters with more than 60% similarity, results mostly corresponding with those of MLST. These results suggest that carbapenem- and aminoglycoside-resistant *K. pneumoniae* isolates producing various combinations of carbapenemase and 16S rRNA methylases are disseminated in medical settings in Nepal. These data indicates that 16S rRNA methylase producing Gram-negative nosocomial pathogen are prevalent in medical settings in Nepal. The project is currently in progress until March 2015.

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 2014
8.	Publications	Molecular epidemiology and antibiotic susceptibility of IMP-type metallo-beta-lactamase-producing *Enterobacter cloacae* isolated in a tertiary medical center in Japan. T. Miyoshi-Akiyama, K. Hayakawa, M. Nagamatsu, K. Shimada, M. Kazuhisa, S. Kubota, E. Kuroda, Y. Sugiki, M. Tojo, N. Takeshita, M. Ujiie, S. Kutsuna, Y. Hamada, N. Ohmagari, T. Kirikae. Presented as poster at ID week, San Francisco, Oct, 2013.
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. The study would include the analyses of the epidemiology of drug-resistant pathogens in Japan, and the comparisons of epidemiological differences between two countries. 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	Oral Presentation: Joint Conference on Infectious Diseases with growing Concern in Recent Years in Nepal, in January 2013.
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 patiernts 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). 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.	

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	Oral presentation 1) 53th Annual Meeting of the Japanese Society of Tropical Medicine, October 2012, Obihiro, Japan 2) 54th Annual Meeting of the Japanese Society of Tropical Medicine, October 2013, Ngasaki, Japan Scientific Paper 1) Fact-finding survey of nosocomial infection control in hospitals in Kathmandu, Nepal- a basis for improvement, Trop Med Heatlh, 41(3): 113-119, 2013 2) Ventilator associated pneumonia in tertiary care hospital, Maharajgunj, Kathmandu, Nepal, J Inst Med, 35(3) 21-28, 2013
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 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 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.

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

Ventilator Associate Pneumonia (VAP) is the most common nosocomial infection among ICU patients and lack of much information in Nepal. This study was conducted 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. A total of 150 tracheal specimens were studied as described by Americal Society for Microbiology. Combination disk method was done for the detection of ESBL and MBL producing isolates.

Prevalence of BAP was found to be 34%. *Acinetobacter calcoaceticus* baumanii complex (44%) was the commonest isolate, followed by *Klebsiella pneumoniae* (22%), *Psudomonas aeruginosa* (16%) and *Staphylococus aureus* (12%). Among MDR Gram negative bacteria, 39% were MBL and 33% were ESBL-producers. Prevalence of MRSA was 75%, which were all sensitive to Vancomycin.

High prevalene of VAP, MDR along with MRSA or ESBL or MBL producing straings was found in this study. Thus, suitable control measures must be adopted to cope with this alarming situation with genetic characterization.

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	
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 2013 the following activities were conducted. 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. Equipment, which is crucial to conduct research and training activities such as desk, chair, computer, projector and scanner, was prepared at the office. 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. Enhancement of human resource development: The following activities were planned or already started. 1) Departments of Research and Microbiology: Hearing on the overall impact of JICA project and current problems was conducted and it found that research methodology, especially, epidemiological methods and diagnostic imaging methods were weak.	

	2) Department of Pulmonology: Regarding the upcoming research entitled “A case study of interstitial pneumonia at Tribhuvan University Teaching Hospital”, NCGM started to give assistance on a creation of research plan as well as give advice on medical imaging diagnosis method (chest X-ray and chest CT) to the department staff. Making an Annual Report The first Annual Report of the IOM-NCGM Collaboration Office was made.

III. Other Activities

1. Joint Conference

The "Joint Conference on Infectious Diseases with Growing Concern in Recent Years in Nepal" was held at IOM (January 18, 2013). A keynote lecture, special lecture, presentations on collaborative research results (seven topics), questions & answers followed by active discussion were conducted. A total of 75 participants (Tribhuvan University counterparts, doctors and nurses from related fields, university teaching staff in Kathmandu, and staff members of JICA Nepal Office) including five doctors from Japan were attended. The program and abstracts are shown on pages 34-47.

Joint Conference at IOM (January 2013)

2. Presentation on Collaborative Research Results

Three counterparts were invited to the 26th and 27th Japan Association for International Health Congress in 2011 (Tokyo) and 2012 (Okayama City) respectively, and the collaborative research results were presented.

In 2013, three counterparts were invited to the 54th Annual Meeting of the Japanese Society of Tropical Medicine (October, Nagasaki City) and one to the 28th Japan Association for International Health Congress (November, Nago City), and the collaborative research results were presented. In spare moments from the annual meeting of the society, a meeting about future research cooperation plans was held by researchers of NCGM and the Nepalese counterparts.

Presentation of the results of collaborative research at Medical Conference in Nagasaki

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. (Cf . pages 27-33)

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 Public Health:
   Regarding the collaborative research with the Department of Public Health entitled "Socio-medical inequities in bed-net use in two different malaria endemicity of Nepal", after conducting the epidemiological analysis and discussion with the department staff, we created the summary for conference presentation. Research results were presented at the 54th Annual Meeting of the Japanese Society of Tropical Medicine (October 2013, Nagasaki City). The results of this study were summarized in a report and soon to be submitted to MOHP, Nepal.
3. 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 January to February in 2014 (24-5). These 2 graduates will continue to do research based on techniques learned, with the goal of obtaining PhD degrees.
4. Department of Internal Medicine, Department of Pulmonology:
   Regarding the upcoming research entitled "A case study of interstitial pneumonia at Tribhuvan University Teaching Hospital", NCGM started to give assistance on a creation of research plan as well as give advice on medical imaging diagnosis method (chest X-ray and chest CT) to the department staff.

Technical guidance to the staff of Departmentof Microbiology, IOM

Collaboration in Internal Medicine

IV. 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.

Table 2

Top 10 cancers in Nepal (both genders out of the total 5041 cases in 2006)

Site of cancer	No. of cases	%
Lung	686	13.6
Cervix uteri	570	11.3
Breast	411	8.2
Stomach	266	5.3
Bone marrow	218	4.3
Unknown primary site	208	4.1
Gallbladder	191	3.8
Colorectum	178	3.5
Ovary	178	3.5
Urinary Bladder	139	2.8

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Table 3

Five leading causes of mortality in Nepal (WHO)

No.	Diseases	%
1	Communicable, Maternal / Perinatal & Nutritional Conditions	42
2	Cardiovascular Diseases	25
3	Cancers	11
4	Injuries	7
5	Respiratory Diseases	5

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Fig. 1

Adult (15-45) HIV prevalence

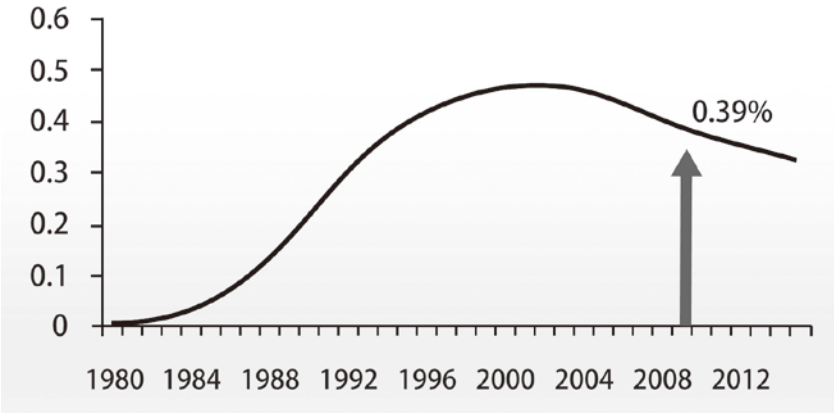
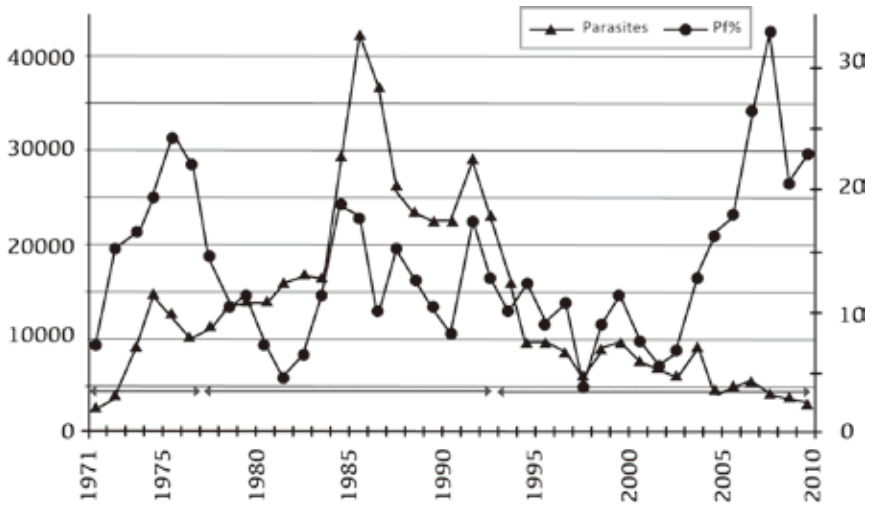


Fig. 2

Transition of the number of malaria cases and rate of falciparum malaria in Nepal



Report from the Ministry of Health and Population in Nepal (2011)

V. Outcome of the Medical Education Project and current IOM/TUTH: a comparative study

1. OVERVIEW OF THE MEDICAL EDUCATION PROJECT:

Medical Education Project (Phase 1 & 2) was a technical cooperation project which had been implemented by Japan International Cooperation Agency (JICA) to Institute of Medicine (IOM) and Tribhuvan University Teaching Hospital (TUTH) from 1980 to 1996.

It was in 1978 that the request for technical cooperation to IOM/TUTH was proposed to the Japanese Government. This was due to the increasing necessity to train medical doctors in Nepal in order to meet the increased demand for the number of doctors and improve the medical situation in Nepal.

After receiving the request preliminary study was conducted. Record of Discussion (R/D) was contracted in June 1980 and project type technical cooperation was started. This project was originally implemented as Tribhuvan University Medical Education Project (Medical Education Project Phase 1) from June 1980 to June 1989 (1985-1988 Extended Period, 1988-1989 Follow-up Period). Following that, the Medical Education Project (Medical Education Project Phase 2) was commenced starting in June 1989. This project was to be completed in June 1994; however it was decided to extend it for a follow-up period until June 19, 1996.

The leading objectives of this project were to establish and substantiate IOM/TUTH as a referral center and core of medical education in Nepal. In Phase 1, it was aimed to establish the basic educational system of both basic and clinical medicine. In Phase 2, consolidation of medical education so as to obtain international approval, upgrading of basic and clinical medicine, enhancement of research level, and improvement of hospital management were designed. The most important purpose of the follow-up period was to smoothly hand over the Project to the Nepal side, reinforcing and strengthening the weak and/or unaccomplished parts of the Project.

Inputs & outputs

The inputs during the 16-year cooperation period (Medical Education Project Phase 1, 2 and Grant Aid) are as follows:

1. Technical Cooperation:

   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

- HMG/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

2. Grant-Aid:
   - 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.

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. 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,

International Symposium on Infectious and Tropical Diseases (1996)

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.

Technical cooperation in hospital management

2. CURRENT IOM/TUTH (IN COMPARISON WITH THE PROJECT END OF TIME):

1. General information and activities

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 4 shows comparison of hospital general information between 1985 and 2013.

2. Clinical Medicine

TUTH is a general hospital which has 750 beds (as of December 2013) 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.

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.

Tribuvan University Teaching Hospital (TUTH)

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.

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 4 Situation and activities of IOM/TUTH : Comparison between 1985 and 2013)

		1985	2013
1	Total No. of beds	401	738
2	No. of charity beds	40	63
3	No. of outpatients served (per year)	229,516	425,163
4	No. of in-patients served (per year)	11,973	19,978
5	Average length of hospitalization (days)	NA	7.27
6	No. of operations (per year)	5,237	11,227
7	Average bed occupancy rate (%)	NA	86.0
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

3. Pulmonology Department: JICA project and thereafter

1. General discussion

During the JICA Project period, though each physician had his or her own subspecialty, department of internal medicine was a single unit without subunit according to specific organs. The only one physician, Dr Rizyal, specialized pulmonology field but the number of the specialists in its field was extremely unbalanced considering the huge burden of the lung diseases in Nepal. The backgrounds of limited candidates for pulmonologists are as follows.

- The results of the sputum examination included smear for tuberculosis bacilli were not reliable.
- Almost all the cases of lung cancer were far-advanced stage and therapeutic intervention was not indicated, thus even making diagnosis of lung cancer was not rewarding process for the doctors. In addition, CT was not available, which is essential for the proper diagnosis.
- Spirometer was not available, which is necessary for diagnosing COPD and bronchial asthma

Nowadays, the quality of various examinations have been improved including sputum examinations, pulmonary function tests, and bronchofiberscope. Accordingly, the number of the doctors in pulmonology department has been increased up to four.

2. Detailed discussion

> Diseases

The most common lung disease for admission is acute exacerbation of COPD. The lung cancer is also common but once diagnosed then the patients will be transferred to Cancer center for the treatment. Others include bronchial asthma, interstitial pneumonitis and so on. Tuberculosis seems to be common in the ward, but without isolation. This structure remains almost the same as in the JICA Project period.

> Bronchoscopy

Before JICA Project, Dr. Prakasa Sayami, thoracic surgeon, was the only one who could practice the procedure. The project intended to increase the number of the bronchoscopists. Nowadays two pulmonologists, Prof. Yogi and Dr. Santa, do it, twice a week.

> Pulmonary function tests

The spirometer, simple and portable one is now available and used for the diagnosis of obstructive lung diseases.

> Clinical study

No clinical studies were conducted before the JICA Project. These days studies have been tried, by part through continuous activities by NCGM supports. Now the joint study is going on. By handling this study, the problems which make conduction of clinical study difficult would be clarified, and the improvement and correction of these problems will be done by first year of this study. One of these issues is the lack of efficient correspondence of the data between Nepal and Japanese institutes, NCGM or Disaster Medical Center, thus internet connection has been established and improved and IT equipments has been introduced. This would continuously facilitate the conductance of future joint study. The statistical analysis for collected data is supported by the Department of Community Medicine and its quality control should also be taken into consideration.

3. Problems and future

DM course is not available in TUTH. The course is established only in Dharan Hospital and Bir Hospital at the moment. As the central medical institute in Nepal, TUTH should also introduce one in the pulmonology Department.

Practical study on respiratory rehabilitation at TUTH

Table 5 Weekly schedule at TUTH

Day	Morning	Afternoon
Sunday	Spirometry + Bronchoscopy + Ward Round	Pulmonology Clinic
Monday	Spirometry + Ward Round	DOTS consultation
Tuesday	General Medicine + Pulmonology (OPD)	Ward Round
Wednesday	Spirometry + Bronchoscopy + Ward Round	DOTS consultation
Thursday	General Medicine + Pulmonology (OPD)	Ward Round
Friday	Spirometry + Ward Round	

Attachment-1

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

Date:	January 18, 2013
Venue:	Institute of Medicine (IOM), Tribhuvan University, Kathmandu, Nepal (Conference hall of IOM at Mohego Building, opposite of research department) (Master of Ceremony: Ms. Shovita Shrestha, Dept. of Microbiology)
8:30	Welcome speech and Introduction of participants: Prof. Jeevan B. Sherchand, Research Director
8:40	Inauguration by lighting the lamp: Prof. Prakash Sayami, Dean, IOM
8:45	Signing the MOU: Prof. Prakash Sayami, Dean, IOM
9:00	Remarks: Prof. Prakash Sayami, Dean, IOM
9:05	Remarks: Prof. Jeevan K. Shrestha, Campus Chief
9:10	Remarks: Prof. Bimal K. Sinha, Asst Dean, IOM
9:15	Remarks: Prof. Bharat M. Pokhrel, Asst Dean, IOM
9:20	Remarks: Prof. Sarala Shrestha, Asst Dean, IOM
9:25	Remarks: Prof. Sharad R. Onta, Asst Dean, IOM
9:30	Remarks: Prof. Bhagawan Koirala, Executive Director, TUTH
9:35	Remarks: Prof. Hiroshi Yoshikura, Advisor, Food and Safety Division, Ministry of Health Labour and Welfare, Japan
(Chair persons:	Prof. Bharat M. Pokhrel, IOM)
9:40 -10:00	***Key note speech:*** Collaboration between IOM/TUTH and NCGM based on the results of the JICA technical cooperation project (Dr. Hiroshi Ohara, NCGM)
10:00 -10:30	***Special lecture:*** Geo-demographic consideration of measles elimination (Prof. Hiroshi Yoshikura, Advisor, Ministry of Health Labour and Welfare, Japan)
Session A:	***Healthcare associated infections*** (Chair persons: Prof. Bharat M. Pokhrel, Dr. Teruo Kirikae)
10:45-11:05	***A-1:*** Fact-finding survey of nococomial infection control in hospitals in Kathmandu (Prof. Bharat M. Pokhrel, IOM)
11:05-11:25	***A-2 :*** Prevalence and bacteriological profile of ventilator associated pneumonia at Tribhuvan University Teaching Hospital (TUTH), a tertiary care hospital, Kathamandu, Nepal (Dr. Ram K. Shrestha, IOM)

11:25-11:45 ***A-3 :*** Intravascular catheter-related infections in tertiary care hospital
(Dr. Jatan B. Sherchan, Kathmandu Univ.)

11:45-12:05 ***A-4:*** Nursing care with special reference to infection prevention & control at Tribhuvan University Teacing Hospital
(Ns. Dharma L. Shrestha)

Session B: ***Multi-drug resistant pathogens***
(Chair persons: Dr. Jatan B. Sherchan, Prof. Hiroshi Yoshikura)

13:00-13:20 ***B-1:*** Emergence of 16S rRNA methallo-beta-lactamase producing nosocomial pathogens in a hospital in Nepal
(Dr. Teruo Kirikae, NCGM)

13:20-13:40 ***B-2:*** Epidemiology on risk factors for isolation of CTX-M type extended- spectrum Beta-lactamase (ESBL) producing Escherichia coli in a large U.S. medical center
(Dr. Kayoko Hayakawa, NCGM)

Session C: ***Vector borne diseases and other infectious diseases with growing concern***
(Chair persons: Prof. Jeevan B. Sherchand, Dr. Shinsaku Skurada)

13:00-13:20 ***C-1:*** Assessment of the interface between malaria control program & general health systems in Nepal
(Dr. Hiroshi Ohara, NCGM)

13:20-13:40 ***C-2:*** Socio-medical study and variation in utilization of bed-nets among different socioeconomic groups of endemic and resurgence areas of Nepal
(Prof. Jeevan B. Sherchand, IOM)

13:40-14:00 ***C-3:*** Study on double burden of tuberculosis and non-communicable diseases in Nepal
(Dr. Shinsaku Sakurada, NCGM)

14:00-14:20 ***C-4:*** Rotavirus gastroenteritis in children under five years of age in Nepal
(Prof. Jeevan B. Sherchand, Dr. Rushika Agrawal, IOM)

14:20-15:00 **Discussion**
(Chair persons: Prof. Bharat M. Pokhrel, Prof. Hiroshi Yoshikura, Dr. Hiroshi Ohara)

15:00 Closing remarks: Prof. Prakash Sayami, Dean, IOM

(Key note speech)

Collaboration between IOM/TUTH and NCGM based on the results of the JICA technical cooperation project

Hiroshi Ohara

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

Tribhuvan University /Institute of Medicine (IOM)/ Teaching Hospital (TUTH) and National Center for Global Health and Medicine (NCGM) have a long history of cooperation starting technical cooperation project supported by JICA. During the project, particularly in the second phase, a lot of experts were dispatched from NCGM and collaborated with TUTH/IOM staff aiming to improve medical and educational situation.

In view of the successful results on the project along with the intimate and reliable relationship, which have been created during the project period and subsequent period, IOM/TUTH and NCGM mutually recognized the importance to continue and develop such precious relationship. After a series of discussion, IOM/TUTH and NCGM agreed to enter into the Memorandum of Understanding (MOU) to promote collaboration between us focusing on researches and related activities.

The overall subject of the collaboration is “Studies on the causative factors and trends of infectious diseases with a growing concern in recent years in Nepal”. Under the above overall subject, sub-researches such as malaria, multi-drug resistant bacteria, infectious diseases & dual burden, nosocomial infection control, diarrheal diseases, hepatitis and so on are to be conducted. We are strongly convinced that such collaboration will actively contribute to promotion of health along with medical science in both countries.

It is a great pleasure that “the Joint Conference on Infectious Diseases with a Growing Concern in Recent Years” was realized in Kathmandu. This conference is jointly organized by IOM/TUTH and NCGM. A total of 12 papers are presented in addition to the special lecture of Prof. H. Yoshikura. I hope this joint conference will be quite successful, our friendly relationship will be tightened and our collaboration is much promoted.

(Special Lecture)

Geo-demographic consideration of measles elimination

Hiroshi Yoshikura

Adviser, Food Safety Division,
Ministry of Health Labour and Welfare

In 2005, World Health Assembly resolution WHA58.15 endorsed a global goal of measles mortality reduction (by 90% compared to the 2000 level) by 2010 or earlier. The reduction of mortality as expressed above could be feasible. However, geo-demographical analysis on the progress of measles elimination in Japan and various WHO regions indicated that attainment of the WHO's numerical indicator of measles elimination by WHO, less than 1 per million population per year, would be very difficult in regions with high population size and density. Geo-demographical consideration is necessary for programming of eradication/elimination of infectious diseases. It may be also true for a small scale program like hospital infection.

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(A-1)

Fact-finding survey of nosocomial infection control in hospitals in Kathmandu, Nepal and trial to improvement

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With the purpose to investigate the actual situation of nosocomial infection control in Kathmandu City, Nepal and contribute to the improvement, a fact-finding survey was conducted targeting 17 hospitals in the city. Survey methods included questionnaire, site visits and interviews. Study items consisted of the infection control system, training situation, surveillance, preparation situation of the personal protective equipment (PPE), existing problems, etc.

Nine out of 17 hospitals had manuals on nosocomial infection control, 7 hospitals had nosocomial infection control committee (ICC), but the meeting was held regularly in only 2 of them. Number of hospitals which met the requited amount of PPE preparation were as follows: Gown (13), Gloves (13), Surgical mask (12). In 6 hospitals in-service training was held during the past 1 year but 7 hospitals responded that no staff had been trained. Surveillance based on the results of bacteriological testing was conducted in 8 hospitals. According to the results in Tribhuvan University Teaching Hospital high frequency of bacterial resistance to antibiotics was shown. Major problems included improper management of ICC, few opportunities to get training among hospital staff, lack of essential equipment. In comparison with the results conducted in 2003 targeting 5 governmental hospitals steady improvement was shown.

In view of the provision of high quality medical care nosocomial infection control in hospitals in Kathmandu needs further improvement. Hereafter it is regarded that widespread of appropriate manual, enhancement of basic techniques, strengthening of the infection control system such as ICC and surveillance are particularly important.

Key words: Fact finding survey, Nosocomial infection control, Kathmandu, Nepal

(A-2)

Prevalence and Bacteriological Profile of Ventilator Associated Pneumonia at Tribhuvan University Teaching Hospital (TUTH), a Tertiary Care Hospital, Kathmandu, Nepal

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In a view to determine the preu, Nepal, from June 2011 to May 2012. One hundred and fifty tracheal aspirates from mechanically ventilated patients were subjected for bacterial culture and their antibiotic sensitivity test at bacteriology laboratory in the Department of Microbiology using standard protocol as described by American Society for Microbiology (ASM). Out of 150 tracheal aspirates, significant bacterial growth was observed in 64 specimens, among which 51 (34%) were associated with VAP. Acinevalence and bacteriological profile of Ventilator Associated Pneumonia (VAP), a study was conducted at Tribhuvan University Teaching Hospital (TUTH), Kathmandtobacter calcoaceticus baumannii complex (n=30, 43.47%), was the most common isolate, followed by Klebsiella pneumoniae (n=15, 21.73%), Pseudomonas aeruginosa (n=11, 15.94%) and Staphylococcus aureus (n=8, 11.59%). Majority of these isolates were multi-drug resistant (MDR) (n=55, 79.7%). Among Gram negative bacteria, 24 (39.33%) isolates were Metallo-β-Lactamase (MBL) producers and 20 (29.5%) isolates were Extended-Spectrum β-Lactamase (ESBL)-producers. Although 47.5% of Gram negative isolates were resistant to Meropenem and Imipenem, however, none of the isolates were resistant to Polymyxin B and Colistin sulphate. Among 8 isolates of S. aureus, 6 (75%) were Methicillin Resistant Staphylococcus aureus (MRSA) which were all sensitive to Vancomycin. These results indicate that MDR along with MRSA or ESBL or MBL producing bacterial strains are prevalent in our Intensive Care Unit (ICU). Therefore, suitable control measures must be adopted to cope up this alarming situation in the hospital.

Key words: Ventilator Associated Pneumonia, MDR, MRSA, ESBL, MBL

(A-3)

Intravascular catheter-related infections in tertiary care hospital

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Introduction: This study had two objectives: 1) to determine the clinical and microbiological profiles of patients developing intravascular catheter-related local (localized catheter colonization and exit site) and systemic infections and their predisposing factors; 2) to study the antibiotic sensitivity patterns of the organisms isolated.

Methodology: This case-control study was conducted over 19 months involving 232 patients at a tertiary care hospital. Non-tunneled central venous catheters and midline catheters were the two types studied. Catheter tips were processed using Maki's roll plate and endoluminal flush techniques. Blood cultures were drawn under strict aseptic precautions and processed by the BacT ALERT system. A "case" was any patient with proven localized catheter colonization, exit site infection or blood-stream infection and a "control" was any patient from whom the intravascular catheter yielded no organism in semi-quantitative cultures.

Results and Conclusions: The incidence of catheter-related blood-stream infections (CRBSI) in our institute was 8.75 per 1,000 catheter days. The commonest organisms causing local infections were coagulase-negative *Staphylococci*, and those causing CRBSI were *Staphylococcus* aureus. Multidrug-resistant organisms accounted for 30.2% of the infections. Risk factors for development of catheter-related infections included an immune compromised state, duration of the catheter *in situ*, femoral venous cannulation, and triple lumen catheters. Choice of venous cannulation to minimize the risk of catheter-related infection in ascending order for risk of infection is the subclavian vein, jugular vein, basilic vein and then the femoral vein. There was no role for empirical antibiotic therapy to prevent intravascular catheter-related local or systemic infections.

Key words: central venous catheters; midline catheters; blood-stream infections; blood cultures

(A-4)

Nursing Care with Special Reference to Infection Prevention & Control at Tribhuvan University Teaching Hospital

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Tribhuvan University Teaching Hospital (TUTH) is a tertiary care health centre of Nepal which was established in 1983 with the support of Japan International Cooperation Agency (JICA). Infection being the major problem in almost all hospitals of Nepal, TUTH has it's own mechanism for prevention & control of infection. This paper intends to share our procedures of infection control at TUTH. This hospital has it's own infection control committee, infection control training curriculum and training program to impart for various categories of heath care workers in the hospital. As a result we have been able to reduce morbidity and mortality rate of patients in the hospital. The author is expecting to have improved procedures for prevention & control of infection in other hospitals of Nepal as well.

(B-1)

Emergence of 16S rRNA methylase producing nosocomial pathogens in a hospital in Nepal

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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 and his colleagues obtained 71 Gram-negative isolates by September 2012, including 17 (24%) *Acinetobactor baumannii*, 16 (23%) *Escherichia coli*, 14 (20%) *Klebsiella pneumonia*, 6 (8%) *Providencia rettgeri*, 4 (6%) *Pseudomonas aeruginosa*, and 3 (4%) *Stenotrophomonas* maltophilia isolates. Of 71 isolates, 26 isolates were highly resistant to all aminoglycosides tested, including amikacin, arbekacin and gentamicin, with MICs of more than 1024 mg/L. Of 26, 21 isolates had 16S rRNA methylase genes, that is known to cause highly resistant to pan-aminogllycosides, i.e., 12 had *armA*, 2 had *rmtB*, 3 had *rmtC*, 4 had isolates had *rmtF*. Remaining 5 isolates were negative for known 8 16S rRNA genes. These data indicates that 16S rRNA methylase producing Gram-negative nosocomial pathogen are prevalent in medical settings in Nepal. The project is currently in progress until March 2015.

(B-2)

Epidemiology and risk factors for isolation of CTX-M type extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* in a large U.S. medical center

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Background: A case-case-control study was conducted to identify independent risk factors for recovery of CTX-M-ESBL-producing *E. coli* (CME-*E. coli*) within a large medical center in Southeast Michigan.

Methods: Unique cases with isolation of ESBL-producing *E. coli* from 2/2010 –7/2011 were included and analyzed by PCR for detection of CTX-M, TEM, SHV β-lactamase gene families. Patients with CME-*E. coli* were compared to patients with non-CME-*E. coli* and uninfected controls.

Results: Of 575 patients with ESBL-producing *E. coli*, 491 (85.4%) tested positive for a CTX-M-ESBL. Three-hundred and nineteen (84.6%) patients with CME-*E. coli* were compared to 58 (15.4%) patients with non-CME-*E. coli*; and the 319 patients with CME-*E. coli* were also compared to uninfected controls. Independent risk factors for isolation of CME-*E. coli* compared to non-CME-*E. coli* included male gender, impaired consciousness, H2 blocker use, immunosuppressive status, and exposure to penicillins and/or trimethoprim/sulfamethoxazole. Compared to uninfected controls, independent risk factors for CME-*E. coli* isolation included the presence of a urinary catheter, history of urinary tract infection, exposure to oxyimino-cephalosporins, dependent functional status, non-home residence, and multiple comorbid conditions. Pathogens were recovered within 48 hours of admission for 77.4% CME-*E. coli* cases and 75.9% non-CME-*E coli* cases. CME-*E. coli* were more resistant to multiple classes of antibiotics than were non-CME-*E. coli*.

Conclusions: CTX-M-encoding genes represented the most common ESBL determinants recovered from ESBL-producing *E. coli*, and the majority of CME-*E. coli* were present upon admission. Regional infection control efforts and judicious antibiotic use are needed to control the spread of ESBL-producing *E. coli*.

(C-1)

Assessment of the interface between malaria control program and general health system in Nepal

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Malaria has been a major public health problem in Nepal. Currently malaria control activities are conducted in 65 out of 75 districts based on the National Malaria Control Program with the support of the Global Fund. In order to implement malaria control effectively, strengthening of health system and integration of the disease specific program into general health system is quite important.

In this study interface between general health system and malaria control program was assessed from the viewpoint of 6 building blocks of health system (Health policy & planning, Service delivery, Workforce, Information system, Medicines & technologies, Financing) proposed by WHO. Based on the framework of analysis, past bottlenecks & good practices to have addressed, current bottlenecks, interactions between general health system and malaria control program were assessed. Furthermore, the assessment results in Nepal were compared with the results of Vietnam and Laos.

Followings were regarded as the leading current bottlenecks in Nepal: ①In some areas imported malaria associated with migration of population and creation of new endemic areas associated with changing environment have been noted. ②Collection of health data from private sector is not adequate. ③Budget for the health programs is still insufficient and sustainability of the programs remains as a challenge. ④Shortage of health manpower is a significant issue at peripheral level. ⑤Long Lasting Insecticidal Nets (LLINs) are not always distributed to the most needed or vulnerable populations. ⑥Quality assurance systems are inadequate.

It is crucial to implement effective malaria control program addressing these bottlenecks toward elimination. Particularly, more efforts are requested to strengthen the health system in remote areas, training of health staff at peripheral level, diagnosis based of accurate quality assurance, promotion of public-private relationship and addressing the issue of imported malaria. These tackling will directly lead to further strengthening of the health system there and eventually effective implementation of various health programs.

(C-2)

Socio-medical Study and variation in utilization of bed-nets among different socioeconomic groups of malaria endemic and resurgence areas of Nepal

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The study examined the variation in utilization of bednets by socioeconomic groups and inequities in access to malaria control services in Nepal. Village Development Committees (VDCs) from four districts were included in which each district with LLIN intervention and two VDCs without intervention from each district were purposively selected. A total of 1050 households from these VDCs were randomly selected and data collected using strutured interview through household survey and Focus Group Discussion. Analysed data reveals the wider disparity and pro-rich inequalities in ownership of bednets. In area without LLIN intervention, bednet ownership was signficantly higher in the rich households where as with LLIN intervention, most of the villagers reported that they did not have barriers to own and utilise bednets as they were getting bednet free of cost. Lack of financial resource was major barrier to obtaining and using bednet in areas without LLIN intervention.

Due to the lack of community-based malaria control activities, villagers were less aware of malaria preventive measures and could not link the health service of HP/SHPs with malaria control services. The results suggest that most poor, excluded and illiterate people are likely to be less benefitted from free net distribution and free healthcare services due to lack of awareness and some deficiencies in the existing programme and services.

(C-3)

STUDY ON DOUBLE BURDEN OF TUBERCULOSIS AND NON-COMMUNICABLE DISEASES IN NEPAL

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While globally the incidence of tuberculosis (TB) has declined gradually, the burden of non-communicable diseases (NCD) has steadily increased in recent decades. There is an increasing trend in developing countries, where the demographic and socio-economic transition imposes more constraints on dealing with the double burden of TB and NCDs in a poor setting. Importantly several NCDs including diabetes mellitus (DM), chronic kidney diseases (CKDs) and alcohol use disorders as well as smoking-related conditions are known to be responsible for a significant proportion of TB cases.

The age group most seriously affected by TB has shifted to elderly population in many developing and even developed countries. There are obviously complications and constraints in finding and treating TB cases in the aged populations, since NCDs have high prevalence among these populations. There is, in particular, a growing evidence that diabetes mellitus is an important risk factor for TB and might affect disease presentation and treatment response. However, the link between TB and other NCDs has not been fully studied yet.

In Nepal the incidence of TB has been declining year by year in recent 5 years, particularly among young population. A slight shift of peak age to elder populations has been observed and it has increased in these years among elder population over 65 years old. We, thus, hypothesize that the age shift may cause complications of TB and NCDs in each individual and inevitably lead Nepali people to the double burden of TB and NCDs.

To verify our hypothesis we have started a pilot study to describe the present stratus over the complication of TB among NCDs and those of NCDs among TB at two clinics in Kathmandu through reviewing patients records, using simple questionnaires to selected patients and conducting focus group discussion of health service staff.

(C-4)

Rotavirus gastroenteritis in children under five years of age in Nepal

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National Center for Global Health and Medicine, Tokyo, Japan

Background: Rotavirus as a causative agent of childhood diarrhea occurs worldwide causing serious illness among children less than 5 years of age. Epidemiological profile, disease burden and diversity of G and P genotypes of rotavirus in Nepal were studied.

Methods: Stool samples were tested for Rotavirus by Enzyme Immuno Assay; strains were genotyped by Reverse-Transcription Polymerase Chain Reaction (RT-PCR).

Results: From January 2009 to December 2011, 2718 cases met the enrollment criteria. The annual enrollment was 906 (33.3%) for 2009, 815 (30.0%) for 2010 and 997 (36.7%) for 2011. Rotavirus was more frequently detected among inpatients (28.5%) than among outpatients (15.2%). Detection was slightly higher among females (25.1%) than males (22.8%). In 2009, rotavirus was most frequently identified in stool specimens collected from children 0 to 11 months of age (28.1%), whereas, in 2010 and 2011, rotavirus was more commonly detected among children 12-23 months of age 35.5% and 26.7%, respectively. Group A rotaviruses were detected by using both ELISA and RT-PCR. Over the three-year study period, 653 (24.4%) cases were positive for rotavirus by ELISA. Genotyping by RT-PCR was completed on 638 samples. The most prevalent genotype was G12P [6] (60.4%). Mixed infections were not uncommon (14% in 2009, 29% in 2010 an 7% in 2011). Forty one samples were partially typed and 23 were completely untyped over the three years study period.

Conclusions: Disease burden and diversity of rotavirus strains circulating in Nepal was studied which would provide useful information to policy makers with regard to introduction of rotavirus vaccine.

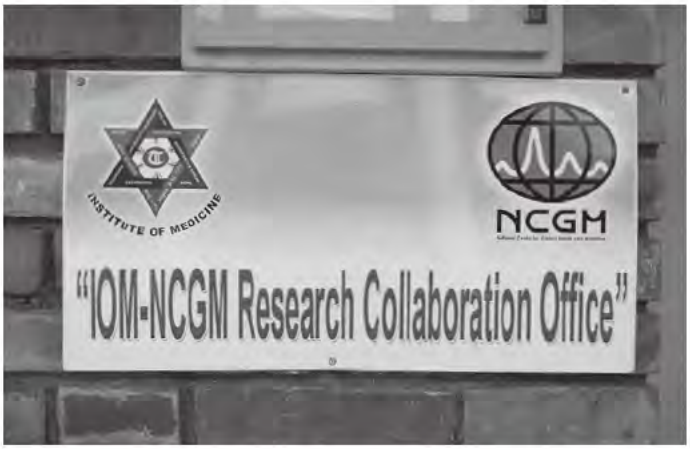
Keywords: Human rotavirus, prevalence, genotype, RT-PCR

Attachment-2

Publications of scientific papers

Papers published on medical journals and abstracts of the report submitted to WHO/WPRO, which were conducted as collaborative study using IOM-NCGM Research Collaboration Office as a base, are shown on pages 51-92 (related important papers in 2011 and 2012 are also included in addition to the papers in 2013).

National Center for Global Health and Medicine (NCGM),
Tokyo, Japan



IOM-NCGM Research Collaboration Office in Academic Building of IOM,
Kathmandu, Nepal

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

Hiroshi Ohara, Shinichiro Noda, Yasuyo Matsumoto, Takanori Hirayama,
Noriko Fujita, Yuriko Egami, Hitoshi Murakami, Hidechika Akashi and Tamotsu Nakasa
Bureau of International Medical Cooperation National Center for Global Health and Medicine (NCGM)

This report is available at http://ncgmimcj.ec-net.jp/HP/library/research_doc/ncgm_report_oct2013.pdf

Executive summary

Global Health Initiatives (GHIs) such as the Global Fund against AIDS, Tuberculosis and Malaria (GFATM) and the Global Alliance for Vaccines and Immunization (GAVI) have supported disease specific programs, resulting in effective control measures in many developing countries. However, there is still some debate regarding the interaction between these programs and the general health systems, as well as some bottlenecks that hinder the smooth implementation of the programs.

This assessment was undertaken with malaria control programs in three countries (Lao PDR, Nepal and Viet Nam) as entry points, in order to assess the interface between malaria control programs supported by GFATM and health systems strengthening, with special reference to interaction between disease specific programs and general health systems. Good practices and bottlenecks in the implementation of the control programs were identified, and possible solutions for these bottlenecks along with synergic health system interventions to be incorporated into disease control programs were discussed.

Primary surveys in this report were conducted in Viet Nam (2009), Lao PDR (2011) and Nepal (2012). In Viet Nam and Nepal the surveys were carried out with special emphasis on best practices and bottlenecks in the implementation of malaria control programs, while in Lao PDR, surveys were carried out on the integration of malaria control programs into the general health systems. In each country, the survey was conducted at various levels (from Ministry of Health to primary level) by document reviews, key informant interviews, and observation of facilities. The results of the primary surveys were analyzed, summarized in reports and submitted to HSD/WPRO. Based on these reports, updating and supplementing more information, and comparative analysis among these three countries, were performed.

In the three countries in this survey, malaria morbidity along with mortality rates were quite high in the past and malaria was given the highest priority in the health policy by the administration. However, since

the mid-1990s, malaria controls were actively implemented in these countries based on the National Malaria Control Programs (NMCPs) and principles of Roll Back Malaria, which consists of strategic priorities including vector control and personal protection, early diagnosis and prompt treatment (EDPT), malaria surveillance and epidemic preparedness, behavioral change communication (BCC), and improving program management. In particular, since early 2000s, GFATM has contributed a large budget to malaria control programs. Recently, malaria in these countries has decreased remarkably; reaching pre-elimination levels (Viet Nam and Nepal) or is no longer listed among from the top 10 diseases (Lao PDR).

In Viet Nam, the government has made great efforts to strengthen the existing health systems since the 1990s (both malaria specific and general health systems) and the international community also cooperated with the policy implementation of Vietnam at various health system levels. Malaria control measures were effectively implemented under the strong leadership of the National Steering Committee, further strengthening and utilizing the existing health system and mobilizing mass organizations.

In Nepal, the general health system, which was fragile in the past, was strengthened and utilized in greater part in the malaria control program. During the period of political instability (1996-2006), health systems were affected, but the influence on malaria control was minimal compared with other disease control programs due to the high governmental priority placed on malaria control and continuous support of the international community.

In Lao PDR, both general health systems (horizontal systems) and specific health systems for malaria control (vertical systems) were weak. After the GFATM intervention, large funds were invested in vertical systems and dissociation from horizontal systems became noticeable. Integration of a malaria control program in the general health system is still limited or partial, and budgetary dependence on GFATM is quite large (97%).

Generally, at upper levels, collaboration among disease specific programs is limited, and a health staff, as well as an infrastructure, is dedicated to each program. However, there is greater integration at lower levels of health care (in Vietnam and Nepal). By coordinating with the community and social organizations in the village, health workers carry out various tasks such as primary health care, implementation of national health programs, preventive medicine, IEC activities, etc. GFATM and other assistant partners provide support by strengthening training and supplying essential medicine and equipment. Malaria control has gradually been more integrated with the primary health care system.

As a result from the surveys in Viet Nam and Nepal, best practices were identified. Among them

intensified education for residents focusing on disease prevention, strengthening of facilities at primary level such as health posts along with training of health workers, utilization of health volunteers at the primary level, high priority attached to frontier areas, and setting up mobile teams, were noteworthy and recognized in common in the two countries. In addition, effective implementation under the strong leadership of National Steering Committee could be seen in Vietnam, utilizing the existing heath system was outstanding. The management system of vertical health programs appeared to have a good impact on the general health system.

In Lao PDR, a survey was conducted focusing on the integration between the malaria control program and general health systems, and the results showed that the extent of integration was quite limited in many elements of program implementation. Particularly, in health information, supply management (procurement, storage, and distribution), and monitoring & evaluation, separate systems were set up between malaria control program implementation and the general health systems. The national health management and information system (HMIS) was considered to be weak, incomplete and unresponsive. Separate financing, governance and planning functions for other disease specific programs, such as Tuberculosis and HIV, distinct from those of the general health system, were also observed.

Bottlenecks/challenges in implementing malaria control were identified in two countries (Viet Nam, Nepal), and points in common included malaria control in frontier areas (many hard-to-reach areas, shortage & low skill of manpower, etc.), low incentives for health workers, a poorly developed reporting system from the private health sector, inadequate quality assurance system for malaria testing (particularly in Nepal). Most health workers are concentrated in large cities and towns, while many health facilities at the primary level (Health Centers; HCs, Primary Health Care Centers; PHCs, Health Posts; HPs, Sub-Health Posts; SHPs) and at some district hospitals the health personnel and/or medical supplies are insufficient. Weak coordination between the local government and GFATM in the distribution of bed nets was pointed out in Nepal. In addition, the current heavy dependence on GFATM undermines the assurance of sustainable malaria control.

Disease specific programs such as malaria control have had some synergic effects on the general health system, particularly at the primary level. The management system of vertical health programs appeared to have a good impact on the general health system at various levels. However, it can be said that dissociation between vertical malaria control program and horizontal general health system also exists. In addition, coordination between malaria control programs and other disease specific programs is limited in many cases. It is true that in implementing malaria control programs, carrying out a vertical health system is important, but scaling up the malaria control program does not automatically lead to general health system strengthening. More effort is needed to realize the maximum synergy between disease specific programs and the general health system, as well as among different health programs.

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Full Length Research

Molecular evidence based hospital acquired rotavirus gastroenteritis in Nepal

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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, molecular, 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

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contact with contaminated surfaces and objects. Cross-infection through contamination of the hands is probably the most common transmission route in healthcare settings. Rotavirus associated gastroenteritis has an incubation period of 1 - 3 days, which is followed by the sudden onset of watery diarrhea, with possible dehydration, vomiting and fever lasting from 4 to 7 days. In the temperate zones of the planet, the virus has seasonal peaks (in the Northern hemisphere from November to March), whereas in tropical regions rotavirus infections occur all year round (Festini *et al.* 2010).

Rotavirus associated gastroenteritis infections contracted by hospitalized children is a source or reservoir of infection, while the transmission route can be toys or other objects which are handled by children, also via the hands of mothers and health care workers which have not been washed properly. The infection contracted in the hospital setting causes the child to stay longer in hospital as well as additional economic and social burden of the guardians/ parents (Stefkovicova *et al.* 2008 and Cunliffe *et al.* 2010). It is important to study prospectively from all admitted children without gastroenteritis symptoms who meet the inclusion criteria for the potential analysis of the incidence of hospital acquired rotaviral gastroenteritis with acceptable precision.

Asymptomatic rotavirus infections are a particular cause of concern. These infections lack the symptoms of vomiting and/or diarrhea, or infected people often show nonspecific symptoms such as fever, headache, nausea, and fatigue (Festini *et al.* 2010; Cunliffe *et al.* 2010 and Cone *et al.* 1988). In the present situation of Nepal, very few studies have been conducted on the incidence of hospital acquired rotavirus infections in pediatric hospitals (Sherchan et al. 2011) and their molecular approaches (Sherchan et al. 2011; Sherchan et al. 2009; Uchida *et al.* 2006; Pun *et al.* 2007; and Shrestha *et al.* 2011). However, no studies have been examined on rotavirus genotyping and hospital acquired infection in Nepal. Hence, the current study attempted to find the incidence of hospital acquired rotavirus gastroenteritis and its molecular evidence among children less than 5 years of age.

METHODOLOGY

The study was conducted in Tribhuvan University Teaching Hospital, Department of Microbiology-Public Health Research Laboratory and Kanti children's hospital, Maharajgunj, Kathmandu. Children under 5 years of age who were admitted without a diagnosis of acute gastroenteritis due to rotavirus and without any clinical symptom suggesting gastroenteritis (diarrhea or vomiting with or without fever) in the periods between January 1, 2010 to 30 December 2010. Written Informed consent was obtained from the children's parents or guardian before enrollment. Recruitment was performed upon hospital admission. From each participating children, clinical data were obtained and first stool sample was collected in a sterile container within 24 hours of hospital admission, regardless of their medical condition. The collected stool samples were tested for rotavirus infection using an antigen detection test by enzyme-linked immunosorbent assay (ELISA) (Premier Rotaclone; Meridian Bioscience, Inc., Cincinnati, OH, USA), according to the instructions of the manufacturer. The participating children whose rotavirus test was positive and those children for whom it was not possible to obtain a stool sample within the same period and in case of withdrawal of consent from the parents were excluded in the study. Children who had negative rotavirus antigen in stool examination on the first day of admission were included in the study. If these children became rotavirus antigen-positive in stool obtained during hospitalization of 48 hours or more or up to 72 hours after discharge, they were considered to have a hospital acquired infection.

For molecular typing genomic RNA was extracted from all rotavirus positive samples which were considered as a hospital acquired infection using the QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and used to determine VP7 and VP4 genotypes by reverse transcription-polymerase chain reaction (RT-PCR) according to described previously (Sherchand *et al.* 2009; Uchida *et al.* 2006 and Gouvea *et al.* 1990).

To determine other enteropathogens associated with hospital acquired infection all stool samples were also examined microscopically for the detection of oocyst, cyst and trophozoites, of protozoa and for larva or eggs of helminthes. A modified Ziehl Neelson staining procedure was used for detecting *Cryptosporidium* species and other coccidian oocysts, including *C. cayetanensis*, from the stool samples. For bacterial identification stool samples were cultured on MacConkey agar (Himedia) for the selection of *E. coli* isolates, Salmonella-Shiegella agar (Himedia) for the selection of *Shigella* and *Salmonella*, and Thiosulfate citrate bile salt agar (Himedia) is selective media for *Vibrio* species. Alkaline peptone water was used as enrichment media for *Vibrio spp.* Purity plate and quality control was maintained through the experimental procedure to maintain the aseptic condition. The cultures were then incubated overnight at 37°C for 24 hrs. All samples were tested for *Vibrio*, *Shigella*, and *Salmonella* by using gram's staining, colony morphology and biochemical properties, and agglutination with specific anti sera.

Analysis

Differences in proportion were assessed by Chi-square test. For comparison between two groups were analyzed by a Mann-Whitney U test (for non parametric data). P values <0.05 were considered statistically significant.

RESULTS

Initially we have recruited 203 children who met the inclusion criteria. Of the 203 children 12 (5.9%) were rotavirus positive by ELISA during hospital admission, even without symptoms of acute gastroenteritis, 25 children were inability to collect stool sample in first 24 hours of admission and 12 children were disappear during follow-up, for which they were excluded. Consequently, 154 pediatric patients in which 98 (63.6%) boys and 56 (36.4%) girls were included in the study. The mean duration of hospitalization was 8 (Range 5-18) days. Of the 154 pediatric patients 31.2% (48/ 154) had positive rotavirus antigen in their stools in the first 72 hours and after discharge and were considered as cases of hospital acquired infection due to rotavirus. Of them, 26 (16.9% were affected during hospitalization, and 22 (14.3%) were affected during 72 hours after discharge. The age wise distribution of hospital acquired rotavirus infection shown in table 1. The highest rate of hospital acquired rotavirus infection 47.8% age between 13 to 24

Table 1. Distribution of hospital acquired rotavirus infection by age groups

Age group (in months)	No. of enrolled children	Rotavirus positive cases (%)	Rotavirus negative cases (%)
03-12	25	5 (20.0)	20 (80.0)
13-24	46	22 (47.8)	24 (52.2)
25-36	31	11 (35.5)	20 (64.5)
37-48	25	6 (24.0)	19 (76.0)
49-60	27	4 (14.8)	23 (85.2)
Total	**154**	**48 (31.2)**	**106 (68.8)**

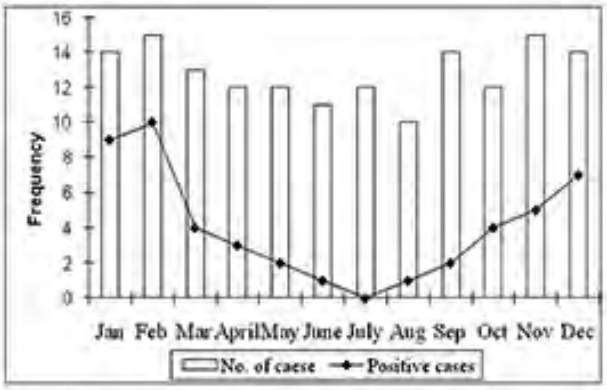

Figure 1. Seasonal distribution of hospital acquired rotavirus infection

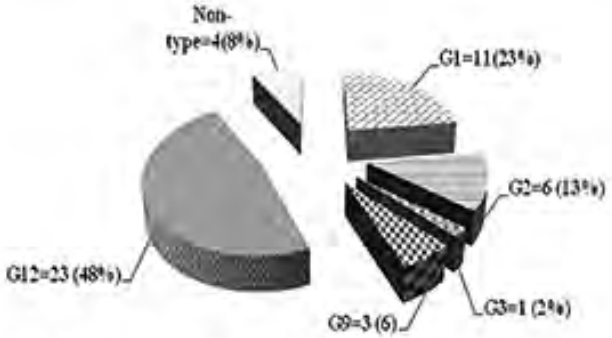

Figure 2. Distribution of G genotypes among hospital acquired rotavirus infected children under 5 years of age

months were significantly difference with other age group ($P < 0.05$).

In addition to the age distribution of hospital acquired infection the seasonality of rotavirus infection was also determined and is depicted in figure 1. Although, the infection found all year round; but the prevalence trend was higher in winter seasons: February 20.8% (10/48) followed by January 18.7% (9/48), December 14.6% (7/48), and November 10.4% (5/48). During the other months, the number of infected cases decreased. July was the heavy rainy season and no cases of rotavirus

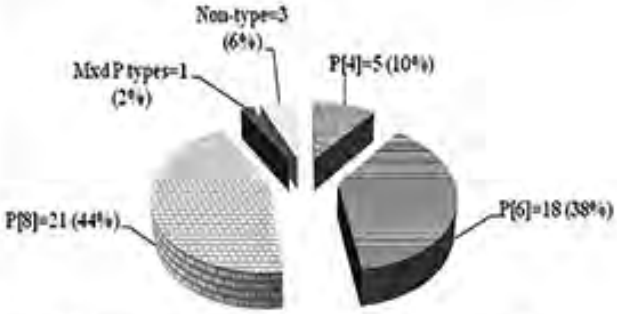

Figure 3. Distribution of P genotypes among hospital acquired

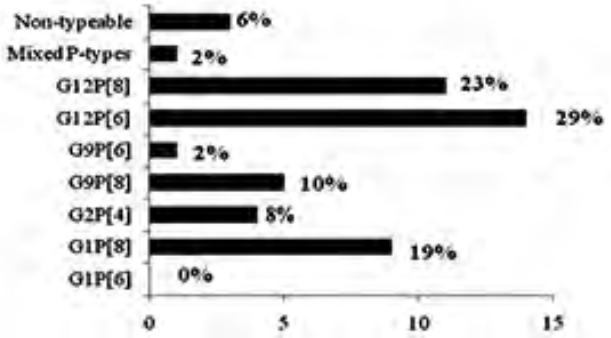

Figure 4. Distribution of G and P genotypes combinations among hospital acquired rotavirus infection in Nepal

were detected, but there was high number of bacterial and protozoal infection was found.

Molecular typing

The molecular evidence of rotavirus genotypes G and P, the predominant serotype G12 represented 48% followed by G1, G2, G9 and G3 of rotavirus strains with 8% non-typeable as shown in figure 2 and was associated with P-types P [8] represented 44%, P[6] 38% and 10% was in P[4] (figure 3). Further, a total of nine G/P type combination were identified, with predominantly G12 P [6] 29% being the most commonly detected rotavirus strain type as depicted in figure 4. Most of the children who had hospital acquired rotavirus gastroenteritis found

Table 2. Distribution of the reasons of hospitalization among the children with hospital acquired rotavirus gastroenteritis; n=48

Reason of admission	No. of cases (%)
Pneumonia	16 (33.3)
Neonatal sepsis (NNS)	8 (16.7)
Abscess	5 (10.4)
Seizure disorder	5 (10.4)
Enteric fever	4 (8.3)
Bronchitis	3 (6.2)
Meningitis	2 (4.2)
Septicaemia	2 (4.2)
Nephrotic syndrome	2 (4.2)
Pleural effusion	1 (2.1)

Table 3: Distribution of hospital acquired enteric-pathogens acquisition in children; n=154

Enteropathogens	No. of cases (%)
Bacteria identification:	24 (15.9)
Escherichia coli (EPEC=7; EAEC=2)	9
Shigella species	5
Vibrio cholera	3
Salmonella species	2
Other pathogens (*Enterobacter=2,klebsiella=1, aeromonas= 1 and pseudomonas=1)*	5
Rotavirus co-infection:	3 (1.94)
Esch coli (EPEC)= 2	2
Shigella species= 1	1
Protozoal parasites	13(8.4)
Giardia lamblia	5
Enamoeba histolytica	3
Cryptosporidium parvum	2
Cyclospora cayetanensis	2
Mixed parasites *(Giardia+Cyclospora cayetanensis)*	1

symptoms of diarrhea, vomiting, fever, poor sucking and dehydration, regardless of the genotype. The study revealed that G 12 and G12P [6] were found major genotypes causing hospital acquired rotavirus gastroenteritis in Nepal.

Distribution of hospitalization children of hospital acquired rotavirus gastroenteritis: In the study children who were suffering from pneumonia were highest (33.3%) followed by neonatal sepsis and the lowest case was children with pleural effusion (2.1%) as depicted in table 2.

Other enteric-pathogens associated with hospital acquired infection

The distribution of hospital acquired enteric-pathogens isolation and identification of bacteria and parasites were shown in table 3 acquisitions in children, 15.9% were pathogenic bacteria and 8.4% were identified protozoal parasites ($p < 0.05$). There were 2% cases of rotavirus co-infection with bacterial pathogens: *Esch coli* (EPEC=2) and one with *Shigella* species. But, there was no rotavirus co-infection with protozoal parasites for causing gastroenteritis.

DISCUSSIONS

Diarrheal disease remains one of the largest health problems in many parts of the world (Bresee *et al.* 2004; Ward *et al.* 2008; and Nguyen *et al.* 2004). Studies in developing countries have shown that children in the first 2 years of the life may have up to 10 episodes of diarrheal disease, often with significant mortality (Nguyen *et al.* 2004; Nepal 2007; and Parasar *et al.* 2003). Diarrheal disease occupied the second place among the top ten diseases in Nepal (Nepal 2007), however, etiologies of diarrhea are diverse with different enteropathogens (Sherchand *et al.* 2011). But in Nepal rotavirus is a leading cause of acute diarrhea in children less 5 years old, accounting for up to 38% of all diarrhea cases (Sherchand *et al.* 2009 and Black 1993). The current study investigated the burden of hospital acquired gastroenteritis from hospitalization children and evidence of rotavirus strain. In our previous study, rotavirus infection was found in 30-50% with acute gastroenteritis from hospitalized and non hospitalized infants and young children (Sherchand *et al.* 2009 and Shariff *et al* 2003). In urban and rural communities of Nepal the burden of rotavirus was 10-15% (Sherchand *et al.* 1992 and

Sherchan *et al.* 2004). Studies conducted in other countries found a prevalence of 8-33% of nosocomial infection (Stefkovicova *et al.* 2008; Cunliffe *et al.* 2010; and Cone *et al.* 1988). The prevalence of rotavirus-associated nosocomial infection reported in Italy was 27.7% (Stefkovicova *et al.* 2008), 14.6% in Australia (Ringenbergs *et al.* 1989), and 19.4% in France (Kirkwood *et al.* 2007 and Pina *et al.* 2000). The relative frequency of this infection is considerably higher in our study than these studies. The methods of these studies were similar to ours, and the differences in the frequencies in different studies may be due to some epidemiologic issues, such as isolation of patients with diarrhea and following health rules in different hospitals.

Our study indicated that the length of hospital stay influences the risk of contracting hospital acquired rotavirus gastroenteritis. In fact, the risk increases in a statistically significant manner after the fifth day in hospital. The strength of our study has twofold, firstly, it is a molecular indicator among hospital acquired rotavirus gastroenteritis children which were carefully monitored and the data collection carried out according to the approved research plan. Secondly, according to our best knowledge, it is the first study of molecular evidence based hospital acquired rotavirus gastroenteritis among pediatric patients in Nepal. However, our study had some limitations. The first limitation was not included from outpatient, which is very important, to identify healthy control, because that provides the real source of infection and rotavirus disease burden. This has been underestimated in many previous studies, which have only included subjects affected by diarrhea during hospitalization, but not outpatient cases. The second limitation was severity of illness, as measured by admission criteria such as pediatric risk of mortality, was not analyzed since it is not routinely recorded in our hospital. Even if it were, its analysis only at admission, as in many hospitals, would not reflect the severity of illness at the time of hospital acquired infection. The third limitation is that the study was conducted in only one hospital, but considering the fact that this hospital is the main referral hospital in Kathmandu valley of Nepal with different subspecialty pediatric wards, the study subjects may be considered to be a good representative sample for the study. The study showed a high frequency of hospital acquired infection due to rotavirus and that most study subjects had acute diarrhea, while others had an asymptomatic infection which is suggested to be due to contamination. These findings emphasize the importance of hospital acquired infections in pediatric wards which, in turn, result in considerable costs of hospitalization and treatment. As documented in some previous studies (Cone *et al.* 1988; Sherchan et al. 2011; and Ringenbergs *et al.* 1989) we suggested that hygienic rules should be followed more strictly in all the wards admitting children.

The contribution of molecular evidence among children with hospital acquired rotavirus gastroenteritis, RT-PCR genotyping showed that G12 strains as the most prevalent Genotype; accounting for one- half of circulating rotavirus detected which was consistent with the preceding studies (Sherchan et al. 2009; Uchida *et al.* 2006; and Pina *et al.* 2000) whereas detection of other G genotypes changed more markedly over time. Similarly, serotype G1 and G9 was the second most common serotype found in the study periods. Serotype G9 has also emerged as one of the common types in other countries during the recent years (WHO 2009 and Potgieter *et al.* 2010). Similarly, of the three different P-types, P[8] was predominant followed by P[6]. Of combined genotypes, G12P [6] was the most prevalent accounting in the study. Although the relative frequency of each of the major G and P combinations of rotavirus strains circulating in Nepal varied in the previous two studies (Sherchan et al. 2009; Uchida *et al.* 2006; and Pun *et al.* 2007) and the current study, G12P[6], G12P[8], G1P[8], G9P[8], G2P[4], and G9P[6] rotavirus were all detected, indicating that there existed a large genetic pool of rotavirus genotypes in Nepal. In addition, the combined genotyping may have advantage in identifying unusual viruses which might help in clarifying the importance of VP4 and VP7 in inducing protective immunity. Unusual G-type and/ or P-type combinations, such as those found in Italy and Australia with G6P [13] specifications (Pina *et al.* 2000 and Potgieter *et al.* 2010) or like those in other countries such as G1P[6] and G9P[6] (Festini *et al.* 2010) were not detected. Recently, (Gentsch *et al.* 1992 and Parasar *et al.* 2003/2006) published data from a "global collection" which includes specimens from the United States, Costa Rica, Korea, Israel, China, Mexico, Bolivia, India, and Bangladesh. These data have shown that the common strain G1P[8] was predominant (53%), followed by G4P[8] (14.3%), G2P[4] (10.7%), G3P[8] (5.4%), strains of mixed genotypes (2.6%), and other genotypes (18.4%). The relatively low frequency of other genotypes or combinations in our study population indicates that the genetic diversity of the Nepalese population of strains is smaller than that reported for the global collection.

Of rotavirus-positive samples from our study about 4% remained untypeable for both G and P types. This could be due to few virus particles with intact RNA in the stool specimens, or the viruses not belonging to genotypes included in the primer set in RT-PCR. Hence, the study emphasized continues and nationwide genotype surveillance of rotavirus is needed to monitor changes in prevalence to identify the emergence of new strains over a time that could affect future vaccination programme of Nepal and to identify any regional differences of genotype prevalence within Nepal.

In this study enteric pathogens acquisition in children with hospital acquired infection was 15.9% (*Esch coli, Shigella* species, *Vibrio cholera, Salmonella species.* Protozoal *parasites* that have been implicated in hospital

acquired infections include *Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum and Cyclospora cayetanensis*. Interestingly, we found 3 children were infected with both rotavirus and either *Esch coli* (EPEC) and *Shigella as* a hospital acquired infection. In one study carried out in China (Ming *et al.* 1991) only one child was reported to be infected with both rotavirus and ETEC. These findings are different from those detected in our study, but, similar with the findings of Tanzania where they reported predominantly diarrhoeagenic *Esch coli* and *Shigella* species co-infection with rotavirus more prevalent in dry season (Moyo *et al.* 2011). However, simultaneous rotavirus and bacterial infections had no significant collaborative influences on clinical symptoms compared to the influences of rotavirus infection or bacterial infection. Furthermore, co-infections could cause difficulties for pediatricians and health care workers for proper diagnosis and treatment of hospital acquired gastroenteritis in children. More studies are necessary in order to evaluate this area further.

CONCLUSIONS

The study demonstrates that hospital acquired rotavirus gastroenteritis was 31.2% which is major public health important among children under 5 years of age. These children can become a source of outbreaks within the hospital and ward as well as their community at home.

The molecular based study revealed that G 12 and G12P [6] were found major genotypes causing hospital acquired rotavirus gastroenteritis in Nepal. So, there is a high priority to extensive study in other areas of Nepal and to introduce of rotavirus vaccine which could substantially reduce morbidity and mortality as well as reduce health care costs in Nepal

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Original article

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

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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

for the management of these devices have decreased their role substantially. Now aspiration of oropharyngeal flora appears to be responsible for most cases of bacterial respiratory infections. Therefore the techniques to alter the flora of the oropharynx and to diminish the risk of aspiration are important priorities for infection control. Exposure to intensive care units (ICUs) is also a major risk factor for nosocomial pulmonary infection and person to person spread of microorganisms within ICUs seems to be responsible for some of these infections[1].

Nosocomial pneumonia is the second most common infection after urinary tract infection and has the highest mortality rate amongst nosocomial infections. Nosocomial pneumonia accounts for 15% of all nosocomial infections and affects 0.5- 2.0% of hospitalized patient. The highest incidence rate was seen in ICU (15-20%) particularly in intubated patients on mechanical ventilation [2].

Almost three quarters of all antibiotic consumptions are for respiratory tract infections [3]. Beta-lactams remain a cornerstone for antimicrobial chemotherapy of a large number of bacterial infections, but their efficacy has been increasingly thwarted by dissemination of acquired resistance determinants among pathogenic bacteria [4]. The exposure of bacterial strains to a multitude of â-lactams has induced a dynamic and continuous production and mutation of â-lactamase in many bacteria, expanding their activity even against later generation cephalosporins [5] and carbapenems by the production of extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) respectively. Since the genes that code for the production of ESBL are often linked to other resistance genes causing extended spectrum of drug resistance, this will result into fewer therapeutic alternatives [6].

According to Clinical and Laboratory Standards Institute (CLSI), once an ESBL producing strain is detected, the laboratory should report it as resistant to all penicillins, cephalosporins and monobactam, even if they test as susceptible in vitro [5]. For improving therapeutic outcomes, reducing the resistance, emergence or prevalence and minimizing costs by limiting and optimizing therapy, respiratory infections are clearly an appropriate area for action.

Carbapenem group of antibiotics play a vital role in the management of hospital acquired gram-negative infections, because of their broad spectrum activity and stability to hydrolysis by most of the â-lactamases, including extended-spectrum â-lactamases (ESBLs). Nosocomial outbreaks of carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. due to metallo â-lactamase production have been reported from different regions [7, 8, 9]. The emergence of these MBLs in gram negative bacilli is becoming a therapeutic challenge as these enzymes possess high hydrolytic activity that leads to degradation of higher generation cephalosporins.

New Delhi strain of MBL producer (NDM-1) gram negatives have become one of the major emerging global threats in recent years [10]. The NDM-1 gene, first identified in Sweden in 2008 in *Klebsiella pneumoniae* from a patient hospitalized in New Delhi, encodes a metallo-ß-lactamase that inactivates all ß-lactams except aztreonam. This bla (NDM-1) gene has been identified in hospital-acquired bacterial species, such as *K. pneumoniae*, but also in the typical community-acquired species, *Escherichia coli*. This gene has been identified in strains that possess other resistance mechanisms contributing to their multidrug resistance patterns. It has been recently extensively reported from the UK, India and Pakistan and, albeit to a lesser extent, from a number of other countries worldwide. In most of the cases a link with the Indian subcontinent has also been established [11].

This underlines the importance of identification of such resistance strains to prevent their dissemination. The resistance mechanisms like ESBL and MBL are already disseminating on a worldwide scale. In recent years MBL genes have spread from *Pseudomonas aeruginosa* to *Enterobacteriaceae* and a clinical scenario appears to be developing that could simulate the global spread of ESBLs. We have also found many carbapenem resistant isolates in the sputum and endotracheal secretion specimens from patients admitted to TUTH.

Due to the lack of antibiotics and disinfecting policies as well as regular monitoring of microbial contamination label among the hospital patients, MDR organisms are believed to have been increased among the hospitalized patients. On the other hand, due to advancement of medical technology, defense mechanisms of the patient's body are bypassed particularly in the patients admitted in the ICU and CCU. This group of patients especially has been found to be victimized by MDR as a result of the immune status of their body. Keeping these in view this study has been subjected to address the issues regarding the burden of MDR as well as ESBL and MBL producing hospital generated lower respiratory tract infection.

Materials and Methods

One hundred nosocomial lower respiratory tract samples including sputum and endotracheal secretion were collected from March 2010 to August 2010, were prospectively studied

in Department of Microbiology, Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal. Specimen collection, culture, identification tests were done according to the guidelines given by American Society for Microbiology. The antibiotic sensitivity test was done by using Mueller-Hinton agar by the standard disk diffusion technique of Kirby- Bauer method as recommended by Clinical and Laboratory Standards Institute (CLSI).

Isolates were labeled as MDR if they were resistant to at least two classes of first line agents including ampicillin, trimethroprim- sulfamethaoxazole, floroquinolones (ciprofloxacin and ofloxacin), Gentamycin and cephalosporins (cefotaxime, ceftriaxone and ceftazidime)[12]. Screening test for the production of ESBL was performed by using ceftazidime (CAZ) (30mg) and cefotaxime (CTX) (30mg) disks. If the zone of inhibition was between d"22 mm for ceftazidime and between d" 27 mm for cefotaxime, the isolate was considered as a potential ESBL producer as recommended by CLSI. The confirmation of ESBL was done by Combination disk method in which CAZ and CTX alone and in combination with clavulanic acid (CA) (10ig) was used. An increase ZOI of e" 5 mm for either antimicrobial agent in combination with CA versus its zone when tested alone confirmed ESBL [13]. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative controls respectively.

Screening for MBL detection was done for the isolates which were resistant to imipenem (IPM 10ug) and meropenem (MEM10ug). The zone of inhibition of 13mm is taken as resistant and 16 mm was taken as sensitive as recommended by CLSI Confirmation was done by combination disk method where two IPM disks (10ig), one containing 10il of 0.1M (292ig) anhydrous EDTA, were placed 25mm apart from centre to centre. An increase in zone diameter of > 4mm around the IMP-EDTA disk compared to that of the IPM disk alone was considered positive for MBL. For MBL test standardization, *P. aeruginosa* ATCC 27853 and *P. aeruginosa* PA 105663 were used as negative and positive controls respectively.

Results

A total of 100 specimens including sputum and ET secretion collected from patients diagnosed of nosocomial lower respiratory tract infection were processed in the Department of Microbiology, TUTH, Kathmandu, Nepal.

Distribution of microbial isolates

Among the total bacterial isolates (n=113), 109 were gram negative and 4 were gram positive.

Distribution of different bacterial isolates

Among the 113 bacterial isolates, majority were *Pseudomonas aeruginosa* (37.2%) followed by *Acinetobacter* spp (31.9 %), *Klebsiella* spp (21.2%), *E. coli* (6.2 %) and *S. aureus* (3.5 %) which is shown in the Fig. 1.

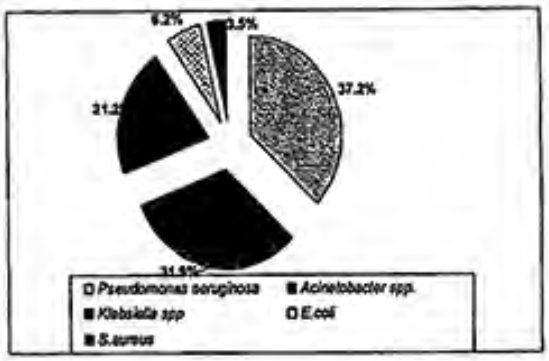

Fig.1. Distribution of different bacterial isolates (n=113)

MDR, ESBL and MBL production in *Pseudomonas aeruginosa*

Out of total 42 *Pseudomonas* isolates, 32 (76.2%) were MDR while equal number was (2.4%) ESBL and MBL producers. All the ESBL and MBL producing isolates were MDR. (Fig. 2)

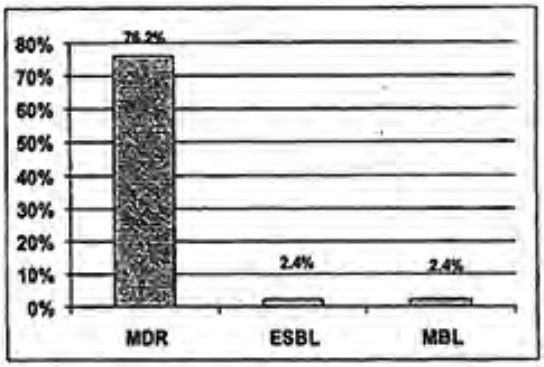

Fig.2. MDR, ESBL, MBL production in *Pseudomonas aeruginosa* (n=42)

MDR, ESBL and MBL production in *Acinetobacter* spp.

Around 97% of *Acinetobacter* isolates were MDR while 47.2% were MBL producers and none were found to produce ESBL which is shown in the fig. 3.

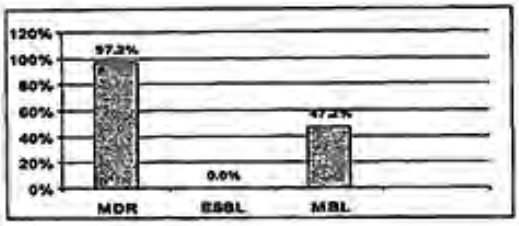

Fig. 3 MDR, ESBL and MBL production in *Acinetobacter* spp.

MDR, ESBL and MBL production in *Klebsiella* spp.

Fig. 4 shows that all *Klebsiella* spp were MDR, 8.3% were ESBL producers and 4.2% were MBL producers.

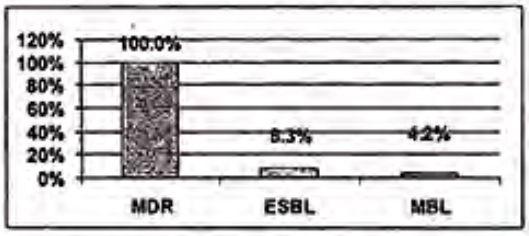

Fig. 4. MDR, ESBL, MBL production in *Klebsiella* spp.

MDR, ESBL and MBL production in *E. coli*

Out of 7 isolates of *E. coli*, all *E. coli* isolates were MDR, 28.6% were ESBL producers, and none were MBL producers.

MRSA

All 4 isolates of *S. aureus* were MRSA and they were sensitive to Vancomycin.

Comparison of MDR, ESBL and MBL production in gram negative isolates.

As shown in figure 5, highest number of MDR and MBL production was in *Acinetobacter* spp. while it was not found to produce ESBL.

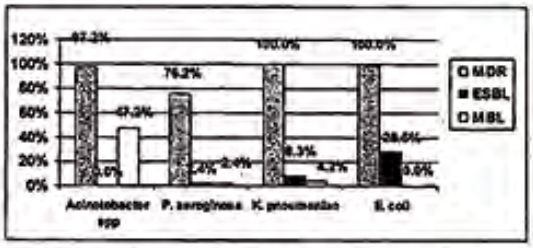

Fig.5. Comparison of MDR, ESBL and MBL production in gram negative bacterial isolates.

Ward wise distribution of MDR, ESBL, MBL and MRSA

Isolates from Intensive care unit (ICU) patient's specimens were found to carry relatively higher frequency of MDR and MBL property. It is followed by Medical Ward (MW), Cardiac care unit (CCU) (Fig. 6).

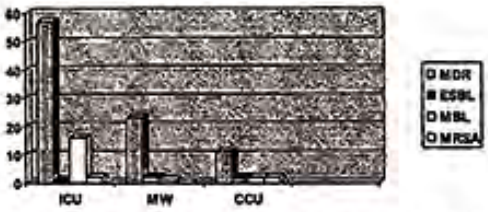

Fig. 6. Ward wise distribution of MDR, ESBL, MBL and MRSA

Discussion

In this study, gram negative bacteria accounted for 96.5% of the total isolates while gram positive bacterial growth was in 3.5% ($P < 0.01$). The most common isolates were found to be *P. aeruginosa* (37.2 %) which has also been found in other studies such as a study by Kampf G et al in Germany [14]. According to Nidhi Goel et al 95.6 % of the isolates were Gram negative and 2.4 % were gram positive cocci. The most common gram negative in order of frequency were *P. aeruginosa* (35%), *Acinetobacter* (23.6%) and *Klebsiella* spp (13.6%) [15]. This correlates with our study which reveals the growth of *P. aeruginosa* (37.1%), *Acinetobacter* spp (31.9 %), *Klebsiella* spp (21.2%) and *E. coli* (6.2%).

Among the bacterial isolates, higher percentage of MDR strains belonged to *Klebsiella* spp (100%) and *E. coli* (100%) followed by *Acinetobacter* and *Pseudomonas*. These pathogens are more common in hospital settings and are mainly accountable for nosocomial infections. Besides, infection by these bacteria is frequently difficult to treat because of both their intrinsic and acquired resistance to multiple groups of antimicrobial agents. Apart from *Klebsiella* spp, MDR isolates were widely present among other genera of *Enterobacteriaceae*. All *E. coli* were MDR. The emergence and increasing trend of MDR among *E. coli* has been reported by others too[16].

According to this study, 12.9% of *Enterobacteriaceae* and 1.3 % of non-fermenters were ESBL producing. Thus ESBL producing isolates are more prevalent among members of *Enterobacteriaceae* ($p < 0.01$). ESBL production is coded by genes that are prevalently located on large conjugative plasmids of 80-160 kb in size [17].Since these plasmids are

easily transmitted among different members of the *Enterobacteriaceae*, accumulation of resistant genes results in strains that contain multi resistant plasmids. So ESBL producing isolates are resistant to a variety of classes of antibiotics. This study showed that all ESBL producers were MDR. Therefore, this study highlights the emergence of ESBL producing strains endowed with extremely wide spectrum of antibiotic resistance.

The decreased susceptibility of gram negative isolates towards the third generation generation cephalosporias-cefotaxime, ceftriaxone and ceftazidime (5-40%) could be attributed to ESBL or Amp C â-lactamase producers or some other relevant underlying mechanisms. This study showed 4.6 % of the gram negative isolates were ESBL producers. ESBL production was most common among *E. coli* (28.6%) followed by *Klebsiella* spp (8.3%) and *Pseudomonas aeruginosa* (2.4%). *Acinetobacter* spp. was not found to produce ESBL. A study by Pokhrel et al in 2004 at TUTH found 24.3% of the total isolates were ESBL producers [18]. They found 55.0% of *K. pneumoniae*, 50% of *E coli* and 20.7% of *Pseudomonas* spp. were ESBL producing. In some hospitals sporadic nosocomial outbreaks due to strains producing ESBLs seem to lead to an endemic problem. Selection pressure from widespread hospital use of later generation cephalosporins apparently enhances colonization of the respiratory tract of patients and infection follows [19,20].

In this study, out of total 113 isolates, 36 were *Acinetobacter* spp. Out of 36 *Acinetobacter* spp. 17 (47.2%) were found to produce MBL. Globally, there has been increasing concern regarding the rise of *Acinetobacter* infection. The infection caused by *Acinetobacter* most frequently involve respiratory tract of intubated patients and *Acinetobacter* pneumonia has been more common in critically ill patients in Asia ranging from 4-44% and European hospitals 0-35%, however it is low in United states Hospitals (6-11%) [21]. Out of 36 *Acinetobacter* isolates, 35 (97.2%) were MDR, 16 were from ICU. The startling rate of MDR *Acinetobacter* underscores the need for cogent step in the treatment option. In the last few years, resistance to antibacterial drugs has been increasing in *Acinetobacter* spp which will likely become a substantial treatment challenge in the future [22]. Carbapenems have potent activity against multidrug resistant *Acinetobacter* isolates. *Acinetobacter* 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 [23,24].

Among the patients under study, 2 were from Medical Ward and another two from CCU that produces ESBL.. There was only one ESBL producer in ICU. These wards comprise the major domicile of ESBL producers. Third generation cephalosporins such as ceftriaxone, cefotaxime and ceftazidime are extremely used in ICUs even in our setting. Therefore the resistance observed here may have appeared under the selective influence of extensive usage of these antibiotics. Moreover, the specific risk factors that apply to ICU patients include the length of hospital stay, the severity of illness, the length of time spent in ICU as well as mechanical ventilation.

In any nosocomial settings, carbapenems are used as the last resort for the treatment of MDR gram negative bacterial infection. However, since last 15 years, acquired resistance to this life saving antimicrobial has been increasingly reported not only in *Pseudomonas* and *Acinetobacter* spp but also among members of *Enterobacteriaceae* [25]. Out of total 109 gram negative bacteria, 17 *Acinetobacter*, 1 *Pseudomonas* and 1 *Klebsiella* spp were found to produce MBL. It consisted of 47.2 % of *Acinetobacter* and 2.4 % of *Pseudomonas* and 4.2 % of *Klebsiella* spp isolates. Out of 19 MBL detected, 16 (84.2%) were from ICU. This is an alarming situation. The MBL producing *P. aeruginosa* in this setting was found to be lower in number than in Bangalore, India (12.0%) [26].

For *Acinetobacter* spp., all the MBL producers were resistant to all the tested first and second line antibiotics except one isolates showed the sensitivity to cotrimoxazole.

Prompt detection of MBL producing isolates is necessary to prevent their dissemination. Carbapenem group of antibiotics play a vital role in the management of hospital acquired gram negative infection, because of their broad spectrum activity and stability to hydrolysis by most of the â-lactamase including extended spectrum â-lactamases (ESBL). Nosocomial outbreaks of carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp due to metallo â- lactamas (MBLs) production have been reported from different regions [7,8,9]. The emergence of these MBLs in gram negative bacilli is becoming a therapeutic challenge as these enzymes possess high hydrolytic activity that leads to degradation of higher generation cephalosporins. Moreover, the treatment alternatives are unavailable or expensive/ toxic with poor outcome [27]. Plasmid mediated MBL genes spread rapidly to other species of gram negative bacilli [28]. Therefore rapid detection of MBL production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination.

New Delhi strain of MBL producer (NDM-1) gram negatives have become one of the major emerging global threats in

recent years [10]. Five multidrug-resistant non-clonally related Enterobacteriaceae isolates were recovered in Belgium in 2010 from 3 patients who had been hospitalised in Pakistan, Montenegro and Serbia/Kosovo. NDM-1 was detected in each of the isolates in addition to several extended-spectrum ß-lactamases (CTX-M-15, SHV-12)[29]. Four *A. baumannii* isolates with bla(NDM-1) were identified in four different provinces in China: no positive isolates were detected among *E. coli, K. pneumoniae* and *P. aeruginosa*. These bla(NDM-1)-positive *A. baumannii* were resistant to all carbapenems and cephalosporins, and three remained susceptible to fluoroquinolones, aminoglycosides and colistin[30]. However, we did not test for specific types of MBL.

Emergence of MBLs producing *Acinetobacter* spp in our clinical strains is alarming and reflects excessive use of carbapenem. Therefore, early detection and prompt instillation of infection control measures is important to prevent further spread of MBLs to other gram negative rods. Additionally, it is also important to follow the antibiotic restriction policies to avoid the excessive use of carbapenem and other broad spectrum antibiotics. To understand the epidemiology, there is a need of genetic analysis and also the typing of metallo- â- lactamases.

In this study there was one *Klebsiella* spp (4.2%) producing MBL from ICU. The proportion of imipenem resistant *Klebsiella* spp. has increased from less than 1% in 2001, to 20% in isolates from hospital wards in Greece and to 50% in isolates from ICUs in 2006 [31]. This situation seems to be due to the spread of the blaVIM-1 cassette among the rapidly evolving multiresistant plasmids and multiresistant or even pan-resistant strains of mainly *K. pneumoniae* and also other enterobacterial species. However the exact biological basis of this phenomenon and the risk factors that facilitates it is not yet fully understood.

Among the MBL producing cases, 84.2% were present in ICU isolates. It has been proved elsewhere that MBL producing *P. aeruginosa* isolates have been reported to be important causes of nosocomial infections associated with clonal spread [32]. The genes for MBL are inserted in integrons and some of these integrons are located on conjugative plasmids. Because of their ability to spread, carbapenem resistance related to MBL production has become a serious concern [33].

Conclusion

In our study, *P. aeruginosa* was found to be the most predominant isolates as a cause of nosocomial lower respiratory tract infections followed by *Acinetobacter* spp, *Klebsiella* spp., *E. coli* and *S. aureus*. This study also showed a very high prevalence of MDR gram negatives among organisms causing nosocomial LRTI. All *Klebsiella* spp and *E. coli* were found to be MDR. Carbapenems and amikacin were found to be the most effective antibiotics for these MDR gram negative bacilli. *Acinetobacter* spp was not found to produce ESBL while they had a high prevalence of MBL. Prevalence of ESBL in *E. coli* was found to be higher, however, no MBL was detected in *E. coli*. MBL producing isolates were more common in ICU.

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KATHMANDU UNIVERSITY MEDICAL JOURNAL

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

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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

opportunistic parasites *Cryptosporidium parvum, Isospora belli* and *Microsporidia* are documented in patients with AIDS.[4,5] Moreover, newly emerging coccidian parasites *Cyclospora cayetanensis* has been reported from HIV-AIDs patients with severe diarrhea in Nepal, India, Peru, Latin America, United States and Papuwa ne Guinea.[6-9] Non opportunistic parasites such as *Entamoeba histolytica, Giardia lamblia, Trichuris trichiura, Ascaris lumbricoides, Strongyloides stercoralis* and *Ancylostoma duodenale* are frequently encountered in developing countries but are not currently considered opportunistic in AIDS patients.[10,11] In immunocompromised patients, the intestinal opportunistic parasites probably play a major role in causing chronic diarrhea accompanied by weight loss.[12] The incidence and prevalence of infection with a particular enteric parasite in HIV/AIDS patients is likely to depend upon the endemicity of that particular parasite in the community.[10] *C. parvum, I. belli* and *E. histolytica* has been reported as the most frequently identified organisms in HIV infected individuals with diarrhea from India and other parts of the world.[9,13-15] HIV/ AIDS infection are rapidly causing a major threat in Nepal. But the study on enteric parasite in HIV/AIDS patients in Nepal is very scarce.[6] Hence the current study was conducted to determine the prevalence of enteric opportunistic parasitic infections among HIV-seropositive patients with and without diarrhea in Kathmandu, Nepal.

METHODS

The study was carried out in Dirgh-Jeevan Health Care Research Center, Tripureswar and Tribhuvan University Teaching Hospital, Public Health Research Laboratory, Kathmandu, Nepal between June 2010 and May 2011. Ethical approval was taken from IRB prior to study. A total of 146 HIV seropositive patients with and without diarrhea participated in the study after giving consent and provided two consecutive stool samples. Before collecting the samples, patient information such as name, age, sex, occupation, clinical history as well as history of diarrhea, antibiotic and antiparasitic treatment history was obtained. Patients already on antiparasitic and antibiotic treatment were excluded from the study.

Blood samples were collected in plain and ethylenediaminetetraacetic acid (EDTA) vials with five ml each from all enrolled patients. Serum samples were used for HIV testing. HIV serostatus of the patients was determined by using commercially available ELISA antibody tests (Genetic system, Biorad Labs, USA and Tridot, J Mitra & Co., India). EDTA blood samples were used for CD4 cell counts and measured by using flow cytometry (Partec, GmbH, Germany). Briefly, 20 µl of CD4 PE antibody was placed in to a Partec test tube and 20 µl of well-mixed whole EDTA blood was added, mixed gently and incubated in the dark for 15 minutes at room temperature. The mixture was agitated during incubation every five minutes. Eight hundred microliters of CD4 buffer was added to the mixture of antibody and sample and mixed gently. This was then plugged to the counter for counting.

Stool samples were collected in clean wide mouthed, leak proof plastic containers from each patient. Stool specimens were examined microscopically for ova, cysts, oocyst, or parasites, using normal saline and iodine mounts on grease-free slides. Following this, each fresh stool samples were preserved in 10% formal saline. The preserved samples were concentrated using formal-ether concentration methods and examined for, Oocyst of *Cryptosporidium spp, Isospora belli*, and *Cyclsopora cayetanensis* were identified using modified Ziehl-Neelsen staining technique earlier described.[9,16,17] The data were analyzed using Chi square (x2) test and appropriate statistical software packages.

RESULTS

The 146 HIV sero-positive patients with and without diarrhea included in the study were aged between 20 to 45 years. Of the 146 patients, the protozoan parasitic infection was found 30.13% (44/146). Of these 146 patients, 78 had diarrhea in which parasitic infection was 39 (50%) as shown in table 1. There was 7.35% (5/68) protozoal parasites positive of cases without diarrhea. A significant difference ($p<0.05$) was observed in the level of infection of intestinal protozoans between the HIV seropositive with diarrhea and HIV-seropositive without diarrhea. Although *Giardia lamblia* and *Entamoeba histolytica* are not considered as opportunistic pathogen it was included in the study because of increased prevalence of these parasites in developing countries.

Table 1.**Distribution of parasitic infection among HIV sero-positive patients.**

Parasitic species	Cases with acute diarrhea n= 33 (22.6%)	Cases with chronic diarrhea n= 45 (30.8%)	Cases without diarrhea n= 68 (46.6%)	Total no. of cases n= 146
Protozoal parasites:				
Giardia lamblia	10	3	1	14
Blastocystis hominis	2	4	3	9
Entamoeba histolytica	2	5	1	8
Cyclospora cayetanensis	1	5	0	6
Cryptosporidium parvum	2	2	0	4
Isospora belli	1	2	0	3
Total	18	21	5	44(30.13%)

Opportunistic parasites and CD4 count

In the study, 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 as depicted in table 2.

Table 2.**Opportunistic parasitic infections and CD4+ T count among HIV sero-positive patients.**

Characteristics	No. Of tested	No. of infection (%)
Gender (HIV patients):	Male: 61 Female: 85	26 (42.6) 18 (21.2)
Clinical symptoms of HIV patients:	Diarrhea: 78	39 (50.00)
	Non-diarrhea: 68	5 (7.35)
CD4 Count (cells/µl) of HIV sero-positive patients	< 200: 43	29 (67.44)
	> 200: 103	15 (14.56)

DISCUSSION

Enteric parasitic infections still remains an important cause of morbidity and mortality in developing countries especially among HIV-infected persons with and without diarrhea.[16] The World Health Organization 2006 defines diarrhea wasting syndrome along with HIV-seropositive patients, the etiology of such diarrhea could either be parasites, bacteria, fungal, enteric virus or HIV itself. [12, 19]

In the present study the enteric parasites were detected in 30.13% from the samples with diarrhea and without diarrhea. There was significant difference the infection of opportunistic parasites among HIV- seropositive cases with diarrhea 50% (39/78) and without diarrhea 7.35% (5/68).

There are number of studies reported from Indian and other countries with the high prevalence of intestinal parasites 25 to 50 % which are near to our findings.[20-22] In the study 43 HIV seropositive patients had CD4 count less than 200 cell/ µl with gastroenteritis parasitic infections and the infection of opportunistic parasites was 67.4% (29/43). Among there parasitic infections *Giardia lamblia* (32%) was predominant pathogens followed by *Blastocystis hominis, Entamoeba histolytica* (18%), *Cyclospora cayetanensis* (14%), *Cryptosporidium spp.* (9%) and *Isospora belli* (7%). Several studies from India and other parts of the world have reported the difference.[23-25] The prevalence of opportunistic parasite in patients with CD4 count less than or equal to 200 cell/µl was found in 14.5%. Cellular immunity is the major defense against intestinal parasitic infections, it is therefore, the reduction in CD4 count by the HIV predispose HIV infected patients to opportunistic intestinal persons to opportunistic infections.[14,26] In our study, CD4 count <200 cells/µl found a significantly higher prevalence of protozoan parasitic infections ($p<0.05$).

C. parvum is a major opportunistic parasitic infection found in other studies.[4, 5, 27] But our study showed low prevalence (9.1%). Similarly *C. cayetanensis* (13.6%) and *I. belli* (7%) were found in the study correlatives with the study done in India.[27] Occurrence of cryptosporidium in both diarrhea and non-diarrhea cases indicates high risk of infection of this parasites in Nepal.

Detection rate of Cyclospora in this study was found to be 13.6% in HIV seropositive patients which does not correlates with the study in India (0.6%) and similar to other study (11%).[27, 28] *Isospora belli* was found (7%) to be predominant cause of morbidity in symptomatic acute and chronic diarrhea. There finding are parallel to those documented in similar studies conducted is different part of world in HIV infected patients.[27,28,29] *Isospora belli* infections are commonly seen in chronic diarrhoeal patients with HIV-AIDS in developing countries ranges 12-20% , *Entamoeba histolyitca* was detected 18% in our study predominant cases with diarrhea should not neglected otherwise.[7,11,22,23,25] Difference in the incidence of intestinal protozoal parasitic infection reported by many researchers can be attributed to the difference in geographical distribution of parasites, sanitary practices, level of education, economic status, social behavior and different selection cases.[14,24,29-31] Although mixed infection is seen in HIV-AIDS patients but in our study we did not observed any such findings. The reason for the same could not ascertain. This could be attributed to the limited study sample and specified place of the country. Moreover, study report on opportunistic parasitic infection among HIV seropositive patients and load of CD4 count in Nepal are very scarce and there is no representative baseline information in the country.[32] Hence, it is important to investigate further to determine the rate of infection with enteric opportunistic parasites to determine the rate of infection with enteric opportunistic parasite in HIV-AIDS patients in other regions of Nepal which will provide the level of endemicity of the country. Skilled manpower and laboratory support required to investigate the carrier, latent and clinical infection. Stool sample examination with modified acid fast staining method as a concentration might help to investigate the existence of opportunistic parasitic infection in Nepal.

CONCLUSION

The study indicated that enteric parasitic infection caused diarrhea (31.13%) of the HIV-seropositive patients. The majority of the infections in the patients with CD4 count < 200 cells/µl were due to enteric opportunistic parasitic infections. The current finding also highlights the importance of early detection of opportunistic parasitic infections among HIV-seropositive patients. This may help to improve the management and quality of life of HIV-infected individuals. Enteric parasites in order to avoid morbidity and mortality due to opportunistic pathogens.

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

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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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TABLE 1 MICs of various β-lactams for *E. coli* strain IMOTU11 and *E. coli* strains transformed with NDM-1 or NDM-8

Antibiotic	MIC (μg/ml)			
	IOMTU11	pHSG398/NDM-8	pHSG398/NDM-1	pHSG398
Ampicillin	>1,024	256	256	4
Ampicillin-sulbactam	>1,024	128	128	2
Aztreonam	>1,024	0.03	0.03	0.03
Cefepime	1,024	0.5	0.5	<0.25
Cefmetazole	ND[a]	4	2	1
Cefoselis	ND	8	4	<0.25
Cefotaxime	>1,024	8	8	<0.25
Cefoxitin	>1,024	64	64	8
Cefozopran	ND	8	8	<0.25
Cefpirome	ND	2	1	<0.25
Cefsulodin	ND	>512	>512	256
Ceftazidime	>1,024	256	256	<0.25
Ceftriaxone	ND	16	32	<0.25
Cefuroxime	ND	512	512	4
Cephradine	>1,024	512	256	16
Doripenem	ND	0.125	0.06	0.03
Imipenem	256	0.5	0.25	0.06
Meropenem	256	0.25	0.5	0.03
Moxalactam	ND	16	8	<0.25
Panipenem	ND	0.5	0.25	0.06
Penicillin G	>1,024	256	256	32
Piperacillin	>1,024	16	16	2
Piperacillin-tazobactam	>1,024	8	8	1
Ticarcillin	>1,024	>512	>512	2
Ticarcillin-clavulanic acid	512	512	512	4

[a] ND, not determined.

tion coefficients for β-lactam substrates have been reported elsewhere (18, 19, 20).

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 (Table 1). The MICs of cefmetazole, cefoselis, cefpirome, doripenem, imipenem, panipenem, and moxalactam were one dilution higher for the *E. coli* strain expressing NDM-8 than for that expressing NDM-1. In contrast, those of ceftriaxone and meropenem were one dilution lower for the NDM-8-expressing strain than for the NDM-1-expressing strain.

As shown in Table 2, recombinant NDM-8 and NDM-1 hydrolyzed all β-lactams tested except aztreonam. The profile of enzymatic activities of NDM-8 against β-lactams was similar to that of NDM-1, although NDM-8 had slightly lower k_{cat}/K_m ratios for penicillin G, ampicillin, cephradine, cefotaxime, and meropenem than NDM-1.

Two amino acid substitutions at positions 88 and 130 slightly affected the enzymatic activities of NDM-8 compared to those of NDM-1 (Table 2). Among all eight NDM variants, amino acid substitutions were found at 6 positions (i.e., positions 28, 88, 95, 130, 154, and 233). It is not yet known which position(s) plays a critical role in the enzymatic activities. The crystal structure of NDM-1 revealed that the active site of NDM-1 is located at the bottom of a shallow groove enclosed by 2 important loops, L3 and L10 (21, 22, 23, 24). Residues 88 and 130, however, were not lo-

TABLE 2 Kinetic parameters of NDM-8 and NDM-1[a]

β-Lactam	NDM-8			NDM-1		
	K_m (μM)[b]	k_{cat} (s^{-1})[b]	k_{cat}/K_m ($\mu M^{-1} s^{-1}$)	K_m (μM)[b]	k_{cat} (s^{-1})[b]	k_{cat}/K_m ($\mu M^{-1} s^{-1}$)
Penicillin G	74 ± 10	91 ± 3	1.20	29 ± 2	79 ± 1	2.70
Ampicillin	193 ± 6	158 ± 5	0.82	122 ± 12	137 ± 5	1.10
Cephradine	52 ± 7	52 ± 4	1.00	37 ± 4	63 ± 1	1.70
Cefoxitin	34 ± 1	3 ± 0.1	0.10	25 ± 6	4 ± 0.3	0.05
Cefotaxime	30 ± 6	38 ± 3	1.30	28 ± 4	45 ± 1	1.70
Ceftazidime	63 ± 3	12 ± 0.2	0.20	74 ± 9	32 ± 2	0.45
Cefepime	153 ± 13	25 ± 1	0.17	152 ± 31	33 ± 5	0.22
Aztreonam	NH[c]	NH	NH	NH	NH	NH
Imipenem	167 ± 8	46 ± 2	0.28	194 ± 38	60 ± 7	0.31
Meropenem	127 ± 20	169 ± 12	1.30	54 ± 10	66 ± 3	1.20

[a] The proteins were initially modified by a His tag, which was removed after purification.
[b] Values are means from three 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.

cated in these loops. These residues may indirectly affect the formation of the active site. NDM-1 may not bind to the carbapenems as tightly as IMP-1 or VIM-2, and it turns over the carbapenems at a rate similar to that of VIM-2 (2). NDM-4 possessed increased hydrolytic activity for carbapenems and several cephalosporins compared to NDM-1 (9). NDM-4 with an amino acid substitution at position 130 (Met to Leu) showed increased hydrolytic activity for carbapenems and several cephalosporins compared to NDM-1 (9). NDM-5 with substitutions at positions 88 (Val to Leu) and 154 (Met to Leu) reduced the susceptibility of *E. coli* transformants to cephalosporins and carbapenems (9). The drug susceptibilities of *E. coli* transformants with bla_{NDM}-2, bla_{NDM-3}, bla_{NDM-6}, and bla_{NDM-7} have not yet been reported. NDM must have only recently started to evolve, and therefore careful monitoring of NDM-producing pathogens is required.

bla_{NDM-8} was found in a plasmid of >100 kb (data not shown). The plasmid was sequenced by using the GS Junior system (Roche Diagnostics K.K, Tokyo, Japan). The sequence surrounding bla_{NDM-8} was *tra*-bla_{NDM-8}-*ble*-*trpF*-*tat*, and the genetic environment of bla_{NDM-8} had more than 99.9% identity at the nucleotide sequence from position 4564 to 8780 bp of *K. pneumoniae* strain GN529 (accession no. HQ416416), which was isolated in Ontario, Canada.

This is the first report describing NDM-1- and NDM-8-producing Gram-negative pathogens in Nepal.

Nucleotide sequence accession number. bla_{NDM-8} has been deposited in GenBank with the accession number AB744718.

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Original article

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

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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

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more recent years, epidemics of novel influenza have also posed a threat of nosocomial infections [9]. These facts made many people realize again the importance of strengthening nosocomial infection control at hospitals in developing countries.

Some of the authors of the present paper have been engaged in technical cooperation for nosocomial infection control with hospitals in developing countries, recognizing the importance of strengthening control measures in order to enhance the quality of medical care. Between 2000 and 2009, they have contributed to the promotion of nosocomial infection control in Vietnam in collaboration with leading hospitals [10, 11].

Since 2010, in response to the growing concern regarding nosocomial infections, we have focused our efforts in Nepal through collaboration with Tribhuvan University Teaching Hospital (TUTH) in Kathmandu City, where a technical cooperation project by Japan International Cooperation Agency (JICA) had been implemented to strengthen the hospital. Following studies including those on hospital-acquired diarrheal diseases [12] and the prevalence of multiple drug-resistant pathogens [13], this survey was carried out as a baseline study aiming to contribute to the improvement of nosocomial infection control at TUTH and consequently in Kathmandu City. The primary purpose of this study was to evaluate nosocomial infection control conditions and to prepare the basic information needed to provide technical guidance.

MATERIALS AND METHODS

1. Fact-finding survey of nosocomial infection control

The subjects of this survey are 17 leading hospitals in Kathmandu City (five national hospitals, nine private hospitals, and three other hospitals). The national hospitals included three general hospitals (one out of three was a university hospital; i.e. TUTH), one pediatric hospital and one obstetric hospital. All the private hospitals were general hospitals, while three other hospitals included one semi-governmental hospital and two non-profit organization hospitals (these three hospitals were general hospitals). The 17 hospitals play a crucial role in medical care in Kathmandu City.

A questionnaire was developed based on the form used in the previous surveys in Vietnam [11]. The form consisted of the following items: "general information of the hospitals, control system including manual and infection control committees (ICC), equipment and facility preparedness, training conditions, surveillance conditions, expectation for international cooperation and current problems. The contents of each item in the questionnaire are shown in Table 1.

The questionnaire was distributed to the 17 hospitals in October 2011 and filled out by the hospital staff members who were responsible for nosocomial infection control or the director of the hospital. The recovered data were processed using SPSS Ver19 for Windows. In some hospitals, to determine the actual situation of ICC, manuals, current problems and awareness level of hospital staff regarding nosocomial infection control, direct observations were conducted along with a brief interview with the hospital staff responsible for nosocomial infection control or hospital di-

Table 1. Questionnare items and contents for 17 hospitals

S.N.	Questionnare items	Contents of questionnaire
1	General information on hospital	Type of hospital, number of beds, number of clinical departments
2	Control system	Existence of nosocomial infection control committee, nosocomial infection control department, infection control team, manual for nosocomial infection control
3	Surveillance conditions	Surveillance according to the report from clinical departments or not, Bacteriological testing on nosocomial infection cases
4	Training conditions	Staff who have received training on nosocomial infection control, Hospitals held in-service training* on nosocomial infection control or not. Hospitals held in-service training on novel influenza or not, Plan of holding "in-service training"
5	Equipment and facility prepredness	Current situation of the preparaton for disinfectants and PPE; Existence of negative rooms, Isolation rooms in the case of novel influenza/SARS, Plan of zoning in the hospital according to the risk of infection
6	Expectations for assistant partners	Hospital wishes to cooperate with foreign assistant partners or not. What cooperation does the hospital expect?
7	Current problems	Requested the hospital to describe the current problems.

* Training organized by the hospital for the staff

rector in addition to the information obtained by the questionnaire.

2. Comparison with the survey results in 2003

In 2003, a questionnaire survey was conducted at five national hospitals in Kathmandu City [10]. These five hospitals were included in this study (2011). The results of the 2003 questionnaire were compared with those of this study (2011), including manuals, ICCs, in-service training and preparedness of personal protective equipment (PPE). A comparative statistical analysis of the 2003 and 2011 results was carried out by the Fisher's exact method using SPSS Ver19 for Windows.

3. Outline of the technical cooperation project and fact-finding survey at TUTH

TUTH was established in 1980 with the assistance of a grant-aid from the Japanese government as the first medical school in Nepal, followed by the implementation of a technical cooperation project supported by the JICA from 1980 to 1996 (the corresponding author participated as a team leader). The purpose of the project was to strengthen medical and educational services at TUTH. During the above period, technical guidance was conducted in the field of hospital management, clinical medicine, nursing management, laboratory management and medical education. However, nosocomial infection control was not included in the project, probably because awareness regarding nosocomial infection control was still poor in those days even in developed countries including Japan. Currently TUTH is playing a leading role in medical care as well as human resource development in Nepal as the oldest and one of the most advanced medical schools.

In this study, the current situation of nosocomial infection control at TUTH was investigated in detail as a basis for further improvement. During the JICA project period, technical guidance was provided, not on nosocomial infection control, but on bacteriological testing as a priority subject. In this study, investigation was performed by direct observation and interviews with heads of the departments of clinical microbiology and pharmacology and doctors of internal medicines, focusing on whether bacteriological testing was utilized for implementation of nosocomial infection control, in addition to detailed observation of the hospital and the questionnaire survey.

4. Ethical approval

Ethical approval was obtained from the Institute of Medicine, Kathmandu, Nepal prior to using the questionnaire in the target hospitals.

RESULTS

1. Fact-finding survey of nosocomial infection control

The 17 hospitals responded to most of the questionnaire items, but for some items, a response was obtained from only 16 hospitals.

General information on hospitals

The average number of beds in the surveyed hospitals was as follows: national hospitals; 372 (150–497), private hospitals; 206 (50–750), other hospitals 328 (156–500). The average number of clinical departments was as follows: national hospitals (excluding the two specialized hospitals); 18.0 (14–22), private hospitals 9.7 (6–15); other hospitals 17.5 (15–20). TUTH, which is one of the national hospitals, had 468 beds and 22 clinical departments.

Control system

Manuals for infection control were used in 52.9% (9/17) of the hospitals (national 4/5, private 3/9, and other hospitals 2/3). However, most of these manuals were more than five years old and some of their contents were not considered suitable for recent infectious diseases and antibiotic use. The manuals at three hospitals were considered obsolete. Two national hospitals had good manuals with up-to-date contents. Only three hospitals had manuals for novel influenza.

An infection control committee (ICC) was established in 41.2% (7/17) of the hospitals (Fig. 1). However, a regular ICC meeting was held in only two hospitals (once a month, and every three months) and the remaining hospitals held meetings when requested. It was noted that the operations of these committees were far from adequate. No hospitals had an infection control team (ICT).

Equipment and facility preparedness:

The number of hospitals which met the standard quantity requirements for disinfectants and personal protective

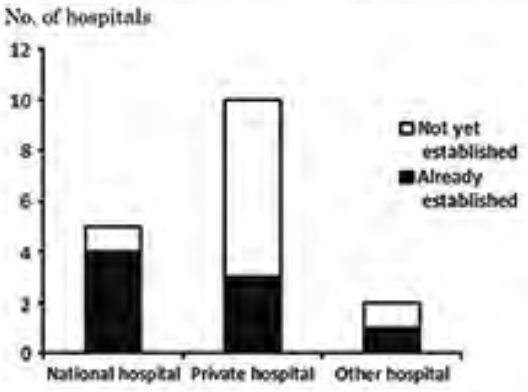

Fig. 1. Hospitals with infection Control Committee (17 hospitals were investigated).

equipment (PPE) is shown in Fig. 2. No hospital was equipped with a sufficient quantity of N95 masks and goggles. Eleven and 12 hospitals responded that N95 masks and goggles were unavailable, respectively.

A total of 81.3% (13/16) of hospitals responded that the preparation level for novel influenza was poor or slight. Four hospitals responded that they could prepare isolation rooms to deal with novel influenza/ SARS, but no hospital was equipped with negative pressure rooms. Only one hospital had a plan of zoning formulated according to the risk of infection.

Training conditions:

Current training conditions are summarized in Table 2. Six hospitals (four national hospitals, one private hospital and one other hospital) were organizing training programs for their staff (in-service training). Regarding future plan, five hospitals responded that they planned to conduct in-service training, and eight hospitals responded that they did not have any plans at the present time but hoped to in the future. Among all the hospitals, one had already conducted a training program on SARS and/or novel influenza and three hospitals intended to conduct training.

Surveillance conditions:

Bacteriological testing was regularly performed for nosocomial infection cases at 62.5% (10/16) of the hospitals and 6.3% (1/16) of the hospitals for some cases. Surveillance of nosocomial infections according to reports from clinical departments on clinical signs such as fever, respiratory signs, diarrhea, etc. was regularly carried out in 43.8% (7/16) of the hospitals in the survey (Fig. 3).

Expectation for international cooperation:

Seven hospitals had a strong interest in cooperating with foreign hospitals. A particularly strong expectation was observed regarding research support, information supply, PPE provision, and guidance in constructing an effective control system (Table 3).

Current problems:

Among the problems observed in the study were weak ICC function, few training opportunities among the hospital staff, inadequate use of antibiotics, shortage of infection control staff, shortage of doctors and nurses and their overload in daily medical practice, shortage of fundamental equipment including PPE, inadequate practice of basic tech-

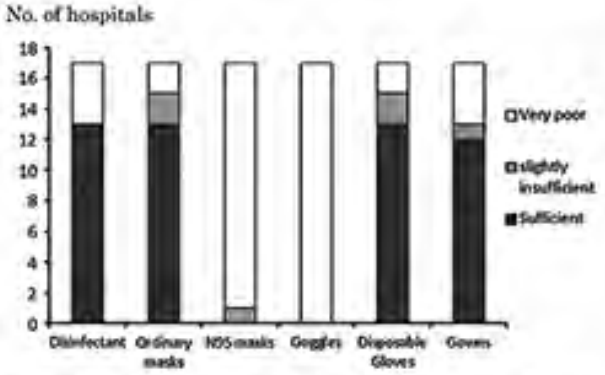

Fig. 2. Hospitals satisfying standard requirements for personal protective equipment (PPE) and disinfectants (17 hospitals were investigated).

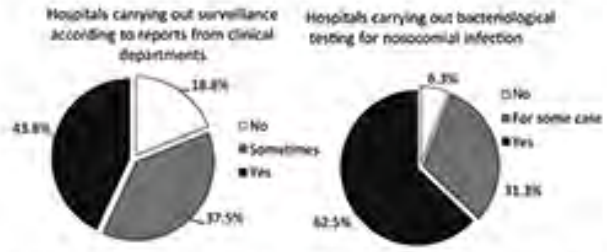

Fig. 3. Surveillance conditions (17 hospitals were investigated).

Table 2. Training activities

Training		No. hospital
Hospital staff received training for nosocomial Infection control	None	7
	A small number	7
	Majority of staff	2
Hospital held in-service training for nosocomial infection control	No	10
	Yes	6
Hospital held in-service training on novel influenza	No	15
	Yes	1

Training: training programs including those organized by other hospitals and the hospital that the staff belong to.
In-service training: training programs organized by the hospital to which the staff belong (16 hospitals were investigated)

Table 3. Expectations for international cooperation

S.N.	Description	No. of Hospitals
1.	Support in research	7
2.	Information provision	6
3.	Supply of personal protective equipment	5
4.	Improvement in the nosocomial infection control system	4
5.	Supply of ICU and emergency equipment	4
6.	Guidance in accepting patients	3
7.	Training for the staff	3
8.	Supply of disinfectants	3
9.	Supply of laboratory equipment	2
10.	Renovation of hospital facilities	2

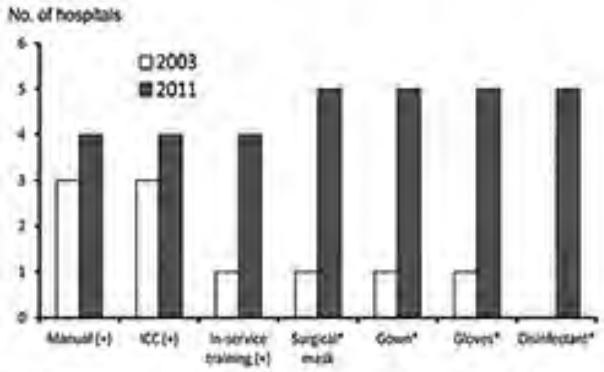

Fig. 4. Comparison of infection control conditions between 2003 and 2011.
* Hospitals with sufficient or nearly sufficient amounts of PPE, disinfectants based on Ministry of Health standards; five national hospitals were investigated.

niques such as standard precautions, inappropriate use of surveillance results, improper disinfection and sterilization methods, and low awareness regarding nosocomial infection control.

2. Comparison of 2003 and 2011 survey results

Comparison of nosocomial infection control conditions between 2003 and 2011 at five national hospitals showed an improvement trend. Particularly, preparation of PPE and disinfectants remarkably improved as shown in Figure 4 ($P = 0.0238$ and $P = 0.004$, respectively), categories in which all five hospitals met the standard quantity. In 2011, four out of five hospitals (except for one specialized hospital) were conducting in-service training, while only one hospital was conducting such training in 2003 ($P = 0.099$). Among these four hospitals, manuals were on hand and an ICC was established ($P = 0.4167$).

3. Nosocomial infection control situation at TUTH

The first ICC in Nepal was established in 1988 at TUTH. Since then, an ICC meeting has been held once a month. A comparatively good infection control manual was prepared and has been revised according to necessity. In-service training has been conducted for most of the staff at TUTH. This study showed a good situation regarding equipment preparedness including disinfectant (sufficient amount), PPE (sufficient amount of ordinary masks, disposable gloves and gowns) along with preparation of isolation rooms. However, incomplete observance of basic techniques such as standard precautions, as well as the need to further strengthen the function of ICC, have been pointed out as challenges.

Performance of bacteriological testing was well carried out in the clinical microbiology department of TUTH, and the results were passed on to the clinical side through the drug information office. However, the interview suggested that increased bacterial resistance to antibiotics was a growing issue at TUTH.

DISCUSSION

Appropriate nosocomial infection control is a key strategy in providing high quality medical care, and effective measures are particularly required in developing countries, where the frequency of infectious diseases is high and environmental conditions of hospitals are poor [14, 15]. However, nosocomial infection control is generally not given high priority, and awareness among medical practitioners is still low, a situation that jeopardizes health care functions.

In this survey in Kathmandu City, steady progress was observed in national hospitals in comparison with the results in 2003. It is particularly noteworthy that awareness among staff and the level of training activities increased with an improvement in the preparedness of essential infection control equipment such as PPE and disinfectants. Regarding private hospitals and other hospitals, a comparative study was not conducted using this survey, but an improvement in infection control similar to that of the national hospitals is assumed.

However, further efforts to strengthen nosocomial infection control at the target hospitals are still considered necessary. The results showed that the majority of hospitals did not have an up-to-date nosocomial infection control manual, that the surveillance system was not established sufficiently, and that preparations against SARS and novel influenza were poor. It is crucial to improve these fundamental systems. Moreover, special emphasis should be placed on observance of basic techniques (standard precautions) such as hand hygiene, effective use of PPE and appropriate practice of disinfection [16–18]. Enlightenment activities, such as distribution of manuals and teaching materials and the organization of training courses for medical staff, are very useful and effective for the improvement of nosocomial infection control. An increasing number of hospitals have been establishing ICCs in recent years, but the management and implementation of activities need further improvement to achieve effective control measures. Hereafter, ICTs also need to be set up in leading hospitals. Furthermore, the detailed status of nosocomial infections and their causative agents should be strictly monitored and properly utilized in clinical practice.

Among the targeted hospitals in this survey, TUTH

showed comparatively good results. Bacteriological testing, supervised by the JICA project, was functioning well and contributing to the surveillance of nosocomial infection based on bacteriological examination and reports from clinical departments for suspected nosocomial infection cases. However, our previous study on pathogens associated with nosocomial lower respiratory infections showed a high frequency of gram negative bacilli such as *Escherichia coli, Pseudomonas aerginosa, Acinetobactor baumanii, Klebsiella pneumoniae*, as well as a high multiple drug resistance rate for isolated bacteria. In addition, a high rate of extended stratum beta lactamase (ESBL) producing bacteria was observed [13]. The spread of multi-resistant bacteria reported by many developing countries is considered to be a facilitating factor in nosocomial infection [19–21]. Methallo β lactamase (MBL) producing bacteria, which originated from India, is also suspected to be spreading to Nepal [13, 22]. These findings suggest the need for more aggressive measures to tackle this global threat. The appropriate use of antibiotics based on accurate bacteriological testing, along with appropriate guidelines, is a worldwide challenge.

Nepal, fortunately, has not experienced a SARS outbreak, and no human case of avian influenza has been reported to date. On the other hand, awareness of nosocomial infection control seems to be lagging behind countries where a SARS outbreak did occur as shown in the 2003 study [10]. When a novel influenza becomes an epidemic and human to human infection is common, nosocomial infections may easily occur as seen in the Spanish influenza pandemic of 1918–1919. Appropriate nosocomial infection control is also considered useful for novel influenza control. Special importance should be placed on setting up a foundation for appropriate nosocomial infection control in daily practice, training medical staff and establishing a control system, before nosocomial infections become a frequent occurrence.

Nosocomial infection control is crucial in providing high quality medical care. Greater efforts should be focused on training medical staff to enhance basic techniques and establish control systems at ordinary times, not waiting until after an outbreak or epidemic. With such a foundation, it will be possible to promptly apply stringent nosocomial infection control in the event of an outbreak of novel influenza, SARS or other emerging infectious disease. These measures will contribute to the reduction of unnecessary costs and can improve the financial condition of the hospital.

Based on the results of this survey, the authors intend to collaborate with Nepalese authorities and further contribute to the improvement of nosocomial infection control. Currently, our collaborative activities at TUTH are related to basic studies on bacterial resistance to antibiotics and the appropriate use of antibiotics. In addition, guidance on the promotion of standard precautions and surveillance systems is currently being prepared. The results of the present survey are expected to provide baseline data for monitoring the progress of the nosocomial infection control situation at TUTH as well as that in hospitals in Kathmandu.

In this survey, only hospitals in Kathmandu City were investigated. Infection control conditions are improving in these hospitals but further improvement in the software aspect is still needed to assure high quality medical care. In Nepal as well as other developing countries, a significant disparity in the conditions of medical care and the health system exists between major cities and rural areas. In the future, the expansion of nosocomial infection control to hospitals in remote areas will be needed along with the implementation of guidance for hospitals in those areas.

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CONFLICT OF INTEREST

None.

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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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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 14138 had >99.9% sequence identity to a nucleotide sequence from nucleotides 65492 to 79611 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 49291 to 58835 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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Ethical approval: This study protocol was ethically reviewed and approved by the Institutional Review Board of the Institute of Medicine, Tribhuvan University (Kathmandu, Nepal) [ref. 6-11-E].

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Original Article

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

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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.

Methods

A Laboratory based Prospective study was conducted in Department of Microbiology, Tribhuvan University Teaching Hospital(TUTH) , Kathmandu from June 2011 to May 2012. This study was approved by Institutional Review Board of Institute of Medicine. Data were analyzed using SPSS, version 17.0. A total of 150 tracheal secretions received for culture

and sensitivity in the laboratory were included in the study. The specimens were cultured on Chocolate agar (CHA), 5% Sheep Blood agar (BA) and MacConkey agar (MA) (Oxoid, UK) plates. On the CHA,bacitracin disk (10 Unit) and optochin disk (5 µg) (Oxoid,UK) were placed at primary and secondary inoculationto screen H. influenzaeand S. pneumoniaerespectively.

The CHA plates were incubated in CO2 incubator (10%CO2) at 37 0C for 24 hours while BA and MA plates were incubated at 37 0C for 24 hours in aerobic atmosphere.

Determination of Bacterial Etiology of VAP:[3]

The etiology of VAP was determined as growth of ≥ 106cfu/ml in endotracheal aspirate and compatible with Gram's stain result of the specimen.

Identification of isolated organisms:

Firstly, pure form of the culture was obtained from the primary culture by using purity plate and then it was processed for different biochemical tests following standard microbiological procedures.

Antibiotic susceptibility testing:

The susceptibility test of the pathogens isolated from the clinical specimens against different antibiotics was done by the standard disk diffusion technique of Kirby-Bauer method as recommended by Clinical and Laboratory Standards Institute (CLSI).4 CHA and BA were used for H. influenza and S. pneumonia respectively to perform sensitivity test. S. aureus ATCC 25923, E. coli ATCC 25922 and P.aeruginosa AT CC 27853 were also tested in every set of experiment, in parallel, as a part of quality control. In this study if the isolates were resistant to at least three classes of first-line antimicrobial agents, they were regarded as MDR.[3]

Tests for ESBL- production in Gram negative isolates[4]

The initial screening test for the production of ESBL was performed by using Ceftazidime (CAZ) (30mg) and Cefotaxime (CTX) (30mg) disks (Mast U.K.). If the zone of inhibition was between ≤22 mm for Ceftazidime and between ≤ 27 mm for Cefotaxime, the isolate was considered as a potential ESBL producer as recommended by CLSI.Confirmations of ESBL producing strains were done by Combination Disc (CD) method in which CAZ and CTX alone and in combination with clavulanic acid (CA) (10µg) were used. An increase ZOI of ≥ 5 mm for either antimicrobial agent in combination with CA versus its zone when tested alone confirmed ESBL.

Tests for MBL-production in Gram-negative isolates[4]

Screening test:

The isolates were subjected for MBL detection when the zone of inhibition (ZOI) was <18mmto imipenem (IPM) (10mg) and/or meropenem (MEM) (10mg). A suspension of bacteria equivalent 0.5 McFarland standard was prepared and was swabbed on to MHA plate.

Combination disk (CD) method:

Two IPM disks (10µg), one containing 10 µl of 0.1 M (292 µg) anhydrous Ethylenediamine-tetraacetic acid (EDTA) (Sigma Chemicals, St. Louis, MO), were placed 25 mm apart(center to center). An increase in zone diameter of >4mm around the IPM-EDTA disk compared to that of the IPM disk alone was considered positive for an MBL.

Tests for MRSA[4]

Thirty microgram cefoxitin disk method as recommended by CLSI was put up and agar plates were incubated at 35°C. The diameter of the zone of inhibition of growth were recorded and interpreted as susceptible or resistant by the criteria of CLSI. S aureus strains ATCC 25923 were used as negative and positive controls respectively. Organisms were deemed methicillin resistant when the zone of inhibition ≤21 mm for S. aureus with cefoxitin disk method.

Results

Number of specimens and result pattern: A total of 150 tracheal secretions were received in the bacteriology laboratory for culture and sensitivity from June 2011 to May 2012. Among the total processed specimens (n=150), significant bacterial growth was found in 64 (42.66%) aspirates, out of which 51(34%) aspirates were associated with VAP as shown in Table 1.

Table 1: Pattern of Tracheal Aspirates Culture result

Growth Pattern	Number	Percent
No growth	86	57.33
Significant growth	64	42.66
Total	150	100
VAP Growth	51	34
Non-VAP Growth	13	9
Total	64	43

Among 51 VAP cases, 37 were of Late Onset type .

Pattern of Bacterial isolates

A total of 69 bacterial isolates were isolated from 51 different tracheal as pirates. Out of 69,61(88.4%) isolates were Gram negative(P<0.01) and remaining (11.59%) were found Gram positive isolates. The commonest isolate causing VAP was Acinetobactercalcoaceticusbaumannii complex(n=30, 43.47%)(P<0.01), followed by Klebsiel lapneumoniae(n=15,21.73%), Pseudomonas aeruginosa (n=11,15.94 %) and Staphylococcus aureus (n=8,11.59%) (Table 2)

Table 2: Pattern of Bacterial isolates from VAP cases (n=51)

Bacterial isolates	Number	Percent
Gram negativebacteria (n=61)		
Acinetobactercalcoa-ceticusbaumanii complex	30	43.47
Klebsiellapnuemoniae	15	21.73
Pseudomonas aeruginosa	11	15.94
Escherichia coli	3	4.34
Citrobacterfreundii	2	2.89
Gram positivebacteria(n=8)		
Staphylococcus aureus	8	11.59
Total	**69**	**100**

Antibiogram of Acinetobactercalcoaceticusbaumanniicomplex

Major VAP isolate i,e., Acinctobacter spp. were found resistant to wide range of antibiotics. Ninety seven percent(n=29) isolates were found resistant to Amikacin and Cefipime, while 83.33%(n=25) isolates were found resistant to Imipenem and Meropenem. However, none of the isolates were found resistant to Polymyxin B and Colistin sulphate.

Antibiogram of Klebsiellapnuemoniae

Klebsiellapnuemoniae, the second commonest isolates were also found resistant to number of antibiotics. Seventy-three percent (n=11)isolates were found resistant to Ciprofloxacin, Ofloxacin, Ceftriaxone and Cefotaxime, and, 67% isolates were found resistant to Amikacin and Cefipime. However, majority of Klebsiellapnuemoniae isolates (n=12, 80%) were found sensitive to Meropenem and Imipenem and all the isolates (n=15, 100%)were found sensitive to Polymyxin B and Colistinsulphate. (Figure 3)

4.19:Antibiogram of Pseudomonas aeruginosa

Antibiotic resistance was also found common in Pseudomonas aeruginosa isolates. Predominant isolates were found resistant to Ciprofloxacin, Amikacin, Cefipime and Piperacillin-tazobactam. However, isolates sensitive to Cefoperazone-sulbactam and Carbapenem were found to be 82% and 91% respectively. Likewise, all the isolates were found sensitive to Polymyxin B and Colistin-sulphate. (Figure 4)

Antibiogram of Staphylococcus aureus(n=8)

Majority of Staphylococcus aureus 6(75%) isolates were found resistant to many antibiotics. However, none of the isolates were found resistant to Vancomycin.

MDR, MBL and ESBL producing Gram negative isolates

Many of the Gram negative isolates were found resistant to number of antibiotics. Eighty percent Gram negative isolates were found to be MDR, which was more common among Acinetobacter,Escherichia and Klebsiella isolates.

Among the MDR Gram negative isolates, 39% were MBL producer and 33% were ESBL producer. MBL producing isolates were most common among Acinetobacter spp. (67%) .

Enterobacteriaceae isolates were found to be common ESBL producer. (Figure 1,2)

Figure 1: Pattern of MDR, MBL and ESBL producing Gram negative isolates

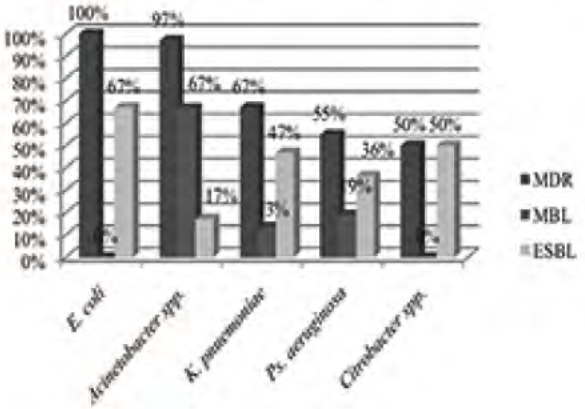
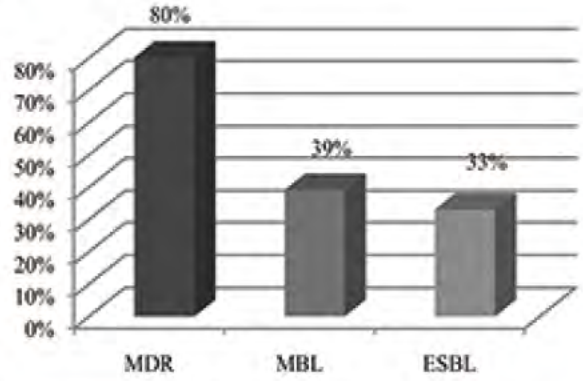


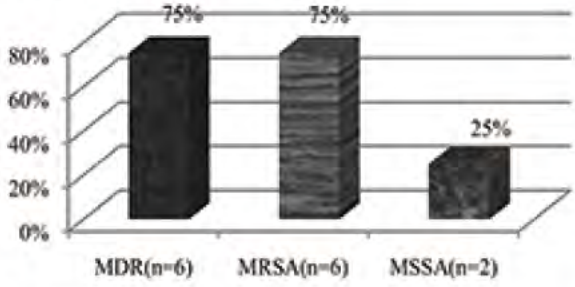
Figure 2: MDR, MBL and ESBL producing Gram negative isolates



Frequency of MDR and MRSA among *Staphylococcus aureus* (n=8)

Out of 8 Staphylococcus aureus, 6 isolates were MDR and MRSA. (Figure 3)

Figure 3: Frequency ofMDR and MRS *Aamong Staphylococcus aureus*(n=8)



Discussion

Among 150 total tracheal aspirates, 64 samples showed significant bacterial growth among which, 51 samples were associated with VAP. In this study, incidence of VAP was found 34% which was similar to findings of Ranjit S etal(31.88%), Dhulikhel hospital,Nepal,2011.[7] Lower incidence of VAP was found in Safdar N et al, (22.8%), 2005, study.[8] Higher incidence of VAP was found among the mechanically ventilated patients in following authors studies; Gadaniet al(37%, Gujarat, India 2010), [9]Dey et al (45.45%),[10]Petdachai W et al (49.4 %,Thailand, 2004), [11]Kanafani ZA et al (47%, Bierut ,Lebnon, 2003), [12]Jakribettu RKP et al (44.2%).[13]

The observation that more than one causative pathogen associated with VAP has been demonstrated in this study. Out of 51 VAP episodes, 35 (68.63%) episodes were monobacterialand 16(31.37%) were mixed bacterial. In study Visscher S et al study,out of 153 VAP episodes 107(69.9%) episodes were monobacterial and 46(30%) were caused by two pathogens.[14]

Significant number of isolates were found Gram negative bacilli(GNB)(n=61, 88.4%) and remaining isolates were Gram positive cocci (GPC) (n=8, 11.59%).(P<0.01) Similar type of findings were found in Koirala P et al (GNB= 89.6%, GPC=10.4%) study from Neuro Hospital, Nepal.[15]In Visscher etal study, 72.37 % isolates were GNB and 27.63% isolates were GPC,[14] and 83% GNB was found in Kanafani ZA et al study.[12]

The aetiological agents associated with VAP in this study were *Acinetobactercalcoaceticusbaumannii* complex, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Citrobacterfreundii*. These isolates were also common in George P et al Manglore, India study.[16] However, more common pathogens were associated with VAP in Visscher S et al study in which *Haemophilusinfluenzae, Streptococcus pnuemoniae,Proteus spp., Enterobacterspp., Serratiasp p.,Morganellamorganii, Stenotrophomonasmaltophilia were additional isolates.*[14]*Burkholderiacepacia* complex was also found common in European and American VAP pathogens.The aetiological agents of VAP vary with different patient populations and types of ICUs.[17] The causative organisms vary with the patients' demographics in the ICU, the method of diagnosis, the duration of hospital stay, and the institutional antimicrobial policies. VAP may be caused by a wide spectrum of bacterial pathogens. Therefore, the local microbial flora causing VAP needs to be studied in each setting to guide more effective and rational utilization of antimicrobial agents.

In this study, non-fermenters(59.1%)were the major pathogens associated with VAP and remaining isolates were enteric gram-negative bacilli (28.96%) and *Staphylococcus aureus* (11.59%). Number of studies show that non-fermenters are major VAP pathogens but their incidence rate varies in different setting and geography. In Trouillet JL et al (France, 1998)[18] study, frequency of non-fermenters and Enterobacteriaceae were 33.9% and 17.9%and in Esperati M et al (Spain, 2010) [19] studies, 28% were Non-fermenters and 26% were enteric Gram-negative bacilli.

Acinetobactercalcoaceticusbaumanniicomplex was the major pathogen responsible for Early Onset as well as Late Onset Type of VAP. (P<0.01).However,in most of the studies, high rates of H.influenza,S. pneumoniae, MSSA, or susceptible Enterobacteriaceae were constantly found in Early Onset VAP, whereas P. aeruginosa, Acinetobacterspp.,

MRSA, and multiresistant GNB were significantly more frequent in Late-Onset VAP e.g Joseph et alstudy.[20] Non-fermenterscolonization during intra-ward admission period before shifting to ICU-MV may be the reason behind Acinetobactersppas common Early Onset VAP pathogens in this study.

Acinetobactercalcoaceticusbaumannii complex (43.47%) was found to be the most common VAP isolate.However, in earlier studies, Pseudomonas spp. used to be the most common ICU pathogens.[15,21] In the global aspect, there has been increasing concern regarding the rise of Acinetobacter infection, ranging from 4-44% in Asian hospitals and 0-35% in European hospitals.[22] These increasing patterns of *Acinetobacter* infection which usually have high mortality rate, has alarmed us that there is further need of extensive study and apply preventive measures to reduce such fearful threat from *Acinetobacter* infection in ICU patients.

In this study, there was high prevalence of MDR Gram negative isolates (80.33%) and Gram positive isolates(75%). Frequency of MDR in Joseph NM et al study was 78.7% (Pondicherry,India, 2010), which was similar to this findings. [20]MDR was found prevalent in all types of bacterial isolates. Out of 30, 29 (96.66%) isolates of *Acinetobactercalcoaceticusbaumannilicomplex* and 10 isolates out of 15 *Klebsiellapnemoniaewere* found MDR. Among 8 *Staphylococcus aureus,* 6 (75%) isolates were MDR. All *Escherichia coli* (n=3/3,100%) isolates were found MDR. As there was relatively less number of *E. coli* isolate as compared with other common Gram negative isolates this figure may not reflect the true scenario. Of the 250 isolates of *Acinetobacter spp.*, 88.4% were MDR in Sweih NA et al study.[23]This study clearly explores that the MDR is common in almost all type of Gram negative as well as Gram positive VAP bacteria complicating the treatment of patients. The emergence of MDR pathogens can be prevented by adopting an antibiotic institutional policy and dose de-escalation regimens.

Drug resistance was found common in the all groups of antibiotics commonly being used. Ninety seven percent(n=29) isolates of Acinetobacter spp. were found resistant to Amikacin and Cefipime, and 83.33% isolates(n=25) were found resistant to Cefoperazone–sulbactam and Carbapenem. In the study conducted by Xie DS et al, China,the frequencies of Imipenem-resistant A. baumannii was 80.3% which was similar to this study.[24]Following resistance frequencies were found in George et al, Manglore, India, study [Amikacin (55.34%), Imipenem (66.67%), Meropenem(75%) and Cefaperazone(75%)].[16]This shows that major VAP isolate resistant to most potent and major reserved drugs.

In this study multi-drug resistance was also found common in *K. pnuemoniae*, the second most common VAP isolate. Isolates resistant to fluroquinolonesand third generation cephalosporins were 73.33 %, to aminoglycolides were 67 % and to Cefoperazone-sulbactamwere 53.33%. Highly resistant Cephalosporin (Cephalexin 75%, Ceftriaxone 85%, Cefotaxime 82.5%)in Amin et al[25] study was similar to my study. However, in this study 20% isolates were resistant to carbapenems. This was similar to George et al study[16],where Klebsiella was found resistant to Meropenem (20%) but higher than Amin et al[25] study, where carbapenams (Imipenem, Meropenem) with the least resistance at 7.5%.

Sixty four *Pseudomonas* isolates were found resistant to fluroquinolones, 54.54% isolates resistant to Amikacin, 45.45 % isolates to Cefipime, 36.36% isolates were found resistant to Piperacillin-tazobactam. However, there was a low resistant frequency (9.1%) to Imipenem/Meropenem/Cefoperozone-sulbactam. In George P et al study,frequencies of Pseudomonasisolates resistance to Amikacin/Piperacillin/Cefoperazone were 14.29%, to Ceftriaxonewere 28.58%, and to Imipenem/Meropenemwere 42.86%.16 In a continuous, prospective, multicentre cohortstudy in Hubei Province, China, by Xie DS et al from January 2007 to June 2009, the frequencies of Imipenem-resistant and Ciprofloxacin-resistant P. aeruginosa were 42.0% and 58.6%respectively.[24]

In this study, Polymyxin B and Colistinsulphate showed excellent effect against all MDR Gram-negative isolates. Except for *Acinetobactercalcoaceticusbaumannii* complex even Imipenem and Meropenem were found to be effective against Gram-negative VAP isolates. Other antibiotics found to be effective were Cefoperazone-sulbactam and Piperacillin-tazobactam in decreasing order for other than *Acinetobactercalcoaceticusbaumannii* complex. Other antimicrobials like Amikacin, Cefipime, Ciprofloxacin, Ofloxacin, and Ceftriaxone showed poor effect among the Gram-negative isolates. High antibiotic resistance rate against commonly used antibiotics is a disadvantage for health care system in countries like Nepal as it can greatly effect patient management. The development of antibiotic resistance is associated with high morbidity and mortality, particularly in the intensive care unit (ICU) setting.

ESBL producing isolates (32.78 %%) were also found common among VAP Gram negative bacteria in this study. They are typically plasmid-mediated clavulanate susceptible enzymes that hydrolyze penicillins, expanded-spectrum cephalosporinsand aztreonam. Among them (32.78%),18% isolates were *enterobacteriaceae* and 14.75% isolates were non-fermenters. Statistically, *Enterobacteriaceaeisolates*

were the commonest ESBL producters. (P<0.01) Among *Enterobacteriaceaeisolates,* 66.66% *Escherichia coli* isolates, followed by *Klebsiellapneumoniae* (53.38%) were found to be ESBL producers. This finding was near about similar to Joseph NM et al (Pondicherry, India) study, where *Escherichia coli* (50%) and *Klebsiellapnuemoniae* (67%) were ESBL producers from VAP cases.[20] However, in Dey et al study, higher frequency 80% of VAP Escherichia coli isolates and 100% of VAP *Klebsiellapneumoniaeisolates* were ESBL producers.[10]

In this study, among non-fermenters, *Pseudomonas aeruginosa* (36.3%) and Acinetobacter spp.(16.66%) isolates were ESBL producers.

Several studies had been carried out at TUTH to determine the prevalence of ESBL among Gram negative isolates. A study conducted by Pokhrel at al in 2004 found 24.27% isolates were ESBL producers and among them 55.0% *K. pneumoniae, 50% E coli and 20.69% Pseudomonas spp.* were ESBL producers among nosocomial and community LRTI isolates.[26] In Mishra SK et al 2008 study ESBL producing isolates were 77.63% in inpatients and 22.37% in out patients.[27] These results shows that there is significant prevalence of ESBL producing isolates causing LRTI in our hospital.

MBL producing isolates (39%) were found more common than ESBL among VAP isolates. Non-fermenters(92%) were significantly predominant MBL producing bacteria compared to enteric bacilli.(P<0.05). Among non-fermenters, Acinetobacter spp. (66.66%) and Pseudomonas aeruginosa(19%)were MBL producing isolates. Acinetobacter isolates were significantly the commonest MBL producer. (P <0.005). Among enteric bacilli, Klebsiellapnuemoniae (33%) isolates were MBL producer. None of E.coli and C. freundiiisolates were found to be MBL producer. The MBL producing P. aeruginosain Joseph NM et al study, (Pondicherry, India) was 20%.20 However,in Dey A et al study,Metallo-β lactamases (MBLs) were produced by 50% of Pseudomonas aeruginosa and 21.74% of Acinetobacter spp.[10]

In Nepal, few studies have been done on the prevalence of MBLs, especially from Hospital isolates. In Mishra et al study, MBL was present in 6 (1.3%) of the total 448 gram-negative isolates.[28] In Shrestha S et al (2010) study at TUTH the prevalence of MBL was 17.43% among nosocomial LRTI, among them *Acinetobacter* (47.22%), *Pseudomonas* (2.38 %) and of *Klebsiellaspp* (4.17%) were MBL producing isolates. Out of 19 MBL producer, 16 (84.21%) were from ICU.[29]

Apart from drug resistance in Gram-negative bacteria, antibiotic resistance has been observed among Gram-positive isolates too. In this study the incidence of methicillin resistant strains among the Staphylococcus aureus isolates in VAP patient was 75%. Variable findings wasobserved in Trouillet JL et al(61.5%, France, 1998), [18]Rodrigues et al (40.7%, Brazil,2009),30 Joseph NM (43%, Pondichery, India,2010), [20] Jones RN (42.5% ,North Liberty, Iowa).[31]Ina continuous, prospective, multi centre cohort study by Xie DS et al of patients who received MV in 17 ICUs in 17 tertiary care hospitals in Hubei Province, China, of all Staphylococcus aureus isolates, 45.7% were methicillin resistant.[24]

VAP MRSA isolates were commonly resistant to antibiotics such as Clindamycin, fluoroquinolones, trimethoprim–sulfamethoxazole. The very low sensitivity of MRSA strains towards Ciprofloxacin, Cotrimoxazole, Erythromycin is probably due to the indiscriminate empirical use of these drugs. The most effective antibiotics for Staphylococcus aureus along with MRSA was found to be Vancomycin, followed by Doxycycline. Although, the Erythromycin resistance against Staphylococcus aureus isolates was very high (75%) in this study, Erythromycin induced Clindamycin resistant case was not found in all the isolated MRSA strain.

From this study, it becomes clear that resistant bacteria are common in our ICU. It is wise to control this situation before it takes a deadly shape. Following the recommendation given by summit on antimicrobial resistance, unnecessary use of antibiotics, identifying the pathogen, choosing correct antibiotics, limiting excess use of antibiotics,improving resistance surveillance systems will help controlling this situation. Although some resistance is inevitable with the use of antibiotics, steps can be taken to curtail practices that cause and propagate resistance. In this way, we will be able to maintain or prolong the efficacy of existing drug.

The incidence of VAP can be prevented by adopting careful intubation techniques, oral tubation, avoiding gastric over- distension, maintaining adequate endo-tracheal cuff pressure and efficient tracheal toileting.[32]

This study helped us in the early diagnosis of VAP and also to determine the incidence of MDR organisms responsible for VAP. The antibiotic susceptibility pattern helped the clinicians to choose the appropriate antibiotics for prophylactic and treatment purposes.

Conclusion

Study shows high prevalence of VAP, MDR along with MRSA or ESBL or MBL producing strains. Thus, suitable control measures must be adopted to cope up this alarming situation with genetic characterization.

Conflict of interest

The authors declare that there is no conflict of interest associated with the study.

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