Annual Report

2017

NCGM-BMH
Medical Collaboration Center

April 2018
Tokyo, Japan-Hanoi, Viet Nam

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Preface

The year 2017 was the eighth year of our intimate collaboration between Bach Mai Hospital (BMH) and the National Center for Global Health and Medicine (NCGM). We, BMH and NCGM, are conducting many activities including the initial stage of "Development of Fundamental International Network for Multi-regional Clinical Trial" in Viet Nam.

This annual report is published to share these results with related authorities and organizations.

We would like to continue our collaboration with both other countries.

Finally, I hope that more fruitful results will be achieved in the next year as well, and I wish that the year 2018 may be good for the people of all over the world, as well as the people of both countries.

April, 2018

Hidechika Akashi, MD, PhD, MPH, DTMH
Director,
Medical Collaboration Center (MCC)
National Center for Global Health and Medicine (NCGM), Japan

Abbreviations

BMH	Bach Mai Hospital
NCGM	National Center for Global Health and Medicine
IMCJ	International Medical Center of Japan
MCC	NCGM - BMH Medical Collaboration Center
MOH	Ministry of Health, Viet Nam
MEXT	Ministry of Education, Culture, Sport, Science and Technology, Japan
J-GRID	Japan Initiative for Global Research Network on Infectious Diseases
MHLW	Ministry of Health, Labor and Welfare, Japan
JICA	Japan International Cooperation Agency
MOU	Memorandum of Understanding
HCMC	Ho Chi Minh City
NIHE	National Institute of Hygiene and Epidemiology
NHP	National Hospital of Pediatrics
NLH	National Lung Hospital
HLH	Hanoi Lung Hospital
NIHBT	National Institute of Hematology and Blood Transfusion
RIT-JATA	Research Institute of Tuberculosis-Japan Anti-Tuberculosis Association
WHO	World Health Organization
JFPIMRC	Japan Foundation for the Promotion of International Medical Research Cooperation
SARS	Severe Acute Respiratory Syndrome
DCC	Disease Control and Prevention Center of NCGM

Contents

I. General information on NCGM-BMH Medical Collaboration Center (MCC)

1. Background

Since the beginning of 1990's, National Center for Global Health and Medicine (NCGM) (former IMCJ) has been carrying out important roles in collaboration with health sector in Viet Nam for the purpose of the improvement of medical situation in the country. Particularly, collaboration with Bach Mai Hospital (BMH) has been implemented most actively and effectively. In the grant-aid and the technical cooperation projects in BMH, which was supported by Japan International Cooperation Agency (JICA), NCGM contributed to the successful implementation by dispatching experts and providing technical guidance.

Through the history of the past collaboration, NCGM has established close and reliable relationship with BMH and other leading medical institutions in Viet Nam. Using these bases, a new collaboration, which was conducted distinctly from ODA projects and focusing on research and human resource development, was designed.

In order to implement the new collaborative activities, the NCGM-BMH Medical Collaboration Center (MCC) was planed.

2. Establishment of MCC

In view of the successful outcome of BMH project (phase 1) and the efficient collaboration during the SARS outbreak in 2003, a plan to establish a medical collaboration center between NCGM and BMH, which functions separately from JICA projects, grew up in NCGM. The idea was put into practice when the research program on emerging and reemerging infectious diseases was proposed by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT).

In recent years, emerging and reemerging infectious diseases have been threatening the world. In view of the rising fear of these diseases, the MEXT launched a new project in world-wide scale to cope with emerging and reemerging infectious diseases efficiently

by setting up medical collaboration centers and conducting close collaboration there. The proposal of the MEXT project facilitated the realization of MCC in Viet Nam. After several preliminary studies, NCGM and BMH decided to establish MCC within BMH based on the friendly and reliable relationship, which had been developed since the beginning of 1990's between the two medical institutions.

The Memorandum of Understanding (MOU) regarding the initiation of the project was signed by the Director of BMH and the President of NCGM in August 2005 followed by the official approval of the Ministry of Health, Viet Nam. In April 2010, NCGM changed its name (from International Medical Center of Japan; IMCJ to National Center for Global Health and Medicine; NCGM) due to its organizational reform (Independent Administrative Legal Entity). In view of this situation, both sides agreed to revise the MOU along with continuation of the current cooperative activities. After discussions between NCGM and BMH the revised MOU was drafted. In the new MOU, activities in MCC are clarified as research, training, medical case conferences, technical cooperation, international conferences/ meetings/ seminars, personal exchange programs, and others, although in the current version description of activity is concentrated on researches. The new MOU, after getting approval of MOH, was signed by the representatives of BMH (Dr. Nguyen Quoc Anh) and NCGM (Dr. Takaaki Kirino) in June 2, 2010.

Signing ceremony of the Memorandum of Understanding between NCGM and BMH in 2010

MCC office was established in the new building of BMH, which was constructed by Japan's grant-aid in 2000, as the managing center of various collaborative activities including the MEXT project and others.

Based on the MCC, various activities were started in collaboration with BMH along with related medical institutions.

3. Objective of MCC

The objective of setting up MCC in Viet Nam is to implement various collaboration on medical science and medical care, such as researches, human resource development & technical exchange, information sharing, clinical case conference, etc. smoothly and effectively. The activities in MCC are conducted in close collaboration between BMH and NCGM, and related medical institutions and such collaborative activities are expected to benefit both Viet Nam and Japan. The contents of activities can include some advanced and sophisticated techniques, which had been difficult to conduct within the framework of JICA projects.

4. Related medical institutions

MCC is mainly collaborating with BMH, however based on the agreement described in MOU; some related medical institutions have been setting up under the approval of BMH.

Currently, five institutions in Hanoi and three institutions in Ho Chi Minh City are functioning as the main related medical institutions. In the future, more medical institutions might be added if necessary and efficient network building among them of are expected.

Table 1: Main medical institutions under collaboration (As of December 2017)

No.	Medical institution	Location	Collaborative study
1	National Institute of Tropical and Infectious Diseases	Hanoi	HIV/AIDS
2	National Lung Hospital (the former National Hospital of Tuberculosis and Lung Diseases)	Hanoi	Tuberculosis
3	Hanoi Lung Hospital (the former Hanoi Hospital of Tuberculosis and Lung Diseases)	Hanoi	Tuberculosis

No.	Medical institution	Location	Collaborative study
4	Cho Ray hospital	HCMC	Healthcare associated infections in Viet Nam
5	Ho Chi Minh Medical and Pharmaceutical University	HCMC	Tropical Medicine
6	Ho Chi Minh City Hospital of Tropical Medicine	HCMC	Tropical Medicine Medical education
7	National Institute of Nutrition	Hanoi	Diabetes and life style related disease

5. MEXT's program

The Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) is implementing the MEXT "Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases" in Asian and African countries. The objective of these activities is to contribute to the emerging infectious diseases and other disease control from the world-wide viewpoint. As of December 2008, this program has been implemented in 11 research centers in 8 countries (Viet Nam, China, India, Thailand, Indonesia, Zambia, Ghana, Philippines). MCC is functioning as one of the important research centers for this program in Viet Nam. The period of this program is 5 years from April 2005 to March 2010. The next step started from April 2010 to March 2015, and now the third step has been started from April 2015.

Currently, the projects supported by MEXT account for the major part of the activities of MCC. Activities of the MEXT projects in Viet Nam include scientific researches (both basic and clinical researches), human resource development, etc. Equipment necessary for conducting these activities has also been provided to BMH and relevant medical institutions.

MEXT's program in Viet Nam consists of the following three research groups (Dr. Shinichi Oka is the leader of these researches). These groups are implementing activities on emerging and reemerging infectious disease control based on the concept of MEXT project. The following three researches are three leading research subjects in MCC and under these research themes, sub-researches have been carried out.

1) Dr. Shinichi Oka's group: HIV/AIDS
2) Dr. Norio Ohmagari's group: Bacterial infections
3) Dr. Naoto Keicho's group: Tuberculosis

6. The program for international promotion of Japan's healthcare technologies and services

The program has been started from 2015, organized by the Bureau of International Health Cooperation

and relevant departments of NCGM and funded by the Ministry of Health, Labour and Welfare, Japan. This program is in relation to the Memorandum of Cooperation in the field of healhcare between the Ministry of Health, Labour and Welfare of Japan and the Ministry of Health of Viet Nam signed on 18 March 2014 by both Ministers. This program aims at carrying out the major objectives of promotion, including sharing the experiences in Japan's public health insurance system and the transfer of cutting-edge medical technologies. Through this program, public health standards in the counterpart countries will be improved.

Under this program, 13 staff members of BMH were invited to NCGM for training in different fields, such as neurosurgery, stroke care, peri-operative care, medical equipment management, hospital management etc. Forty-four experts from NCGM also came to BMH for investigating the needs, discussing the contents of training and collaborative activities, as well as following-up the implementation in BMH after training courses. This program was highly evaluated by BMH, contributing to provide better healthcare services to more people in Viet Nam.

Healthcare staff working in quality management and patient safety in BMH (one staff) and other related hospitals (six staff members) were also invited to NCGM for training. In September 2017, the 3rd Viet Nam Forum on Quality Management and Patient Safety was held in Ho Chi Minh City and Ba Ria City located in the South of Viet Nam. The participants joined in hospital tours in Hung Vuong Hospital and Thu Duc Hospital to learn from these hospitals' actual experiences prior to the two-day discussion forum held in Ba Ria Hospital, Ba Ria City consisting of 241 participants.

7. Other projects

In addition to the obove, the Ministry of Health, Labor and Welfare of Japan (MHLW) is also supporting research projects on various fields. As an oversea base of NCGM, MCC is functioning as a body to support NCGM research teams or individuals who want to implement a collaborative project in Viet Nam.

Within this scope, life-style related diseases such as diabetes and pediatric health issues are projects which have been implemented in collaboration with BMH and other medical institutions of Viet Nam so far. A community-based suvery on diabetes and obesity, followed by an intervention program has been implementing in Hanoi area and a lot of meaningful data have been obtained with the support from MCC.

In 2013, the Sister Renal Center Program was applied in cooperation between Nephro-Urology Department, BMH and Nephrology Department, NCGM to receive the support from the International Society of Nephrology. Under this program, in 2017, Japanese groups including NCGM nephrologists and medical engineers came to BMH for training courses on kidney diseases and quality management of water for hemodialysis, with the trainees from BMH and other hospitals. Together with the Bureau of International Medical Cooperation

of NCGM, MCC participated in supporting the implementation of this program.

NCGM delegation attended the 2017 Annual Meeting of Hospitals' Directors of the Northern Provinces held in Lao Cai province, Viet Nam

8. Current MCC

In 2017, MCC received the NCGM delegation leaded by the President of NCGM, Dr. Norihiro Kokudo. The delegation visited BMH and attended the Annual Meeting of Hospitals' Directors of the Northern Provinces of Viet Nam. The Minister of Health of Viet Nam also attended this meeting and a lot of experiences have been exchanged.

MCC also received many groups of researchers and specialists coming from NCGM and related institutions in Japan. MCC staff participated in discussions on related activities held between the two sides. Together with Vietnamese counterparts, MCC staff took part in conducting, supervising and monitoring on-site implementation of researches, as well as collecting and reporting data. In addition to that, MCC has provided logistic support to these groups, such as on-site coordination including making appointments with Vietnamese counterparts, reservation of accommodation, arrangement of transportation vehicles etc.

In 2017, MCC also participated in arrangement of the training visits for NCGM trainees. Trainee groups including doctors, nurses, technicians, and pharmacists came to BMH and other hospitals and healthcare centers in Hoa Binh province for the training on global health and medicine.

MCC staff also supported administrative procedures for Vietnamese counterparts, who were invited to Japan for training. In 2017, more than 30 counterpart members have completed necessary procedures and successfully received training in Japan. Through this activity, MCC also acts as a bridge to link domestic health personnel and institutions, which is necessary for sustainable improvement.

II. Activities

1. Research

List of collaborative researches in MCC, Viet Nam

Table 2 : Collaborative researches in MCC, Viet Nam

No.	Main Researchers	Affiliation in Viet Nam	Subject	Source of fund
1	Shinichi Oka (NCGM)	National Hospital of Tropical Diseases (NHTD) Bach Mai Hospital(BMH)	The cohort study of HIV-1-infected individuals in Northern Viet Nam	AMED
2	Kajio H (NCGM) Pham MT (BMH) Nguyen KDV (BMH)	Bach Mai Hospital (BMH)	Study on the contribution of obesity to diabetes and blood vascular diseases in Viet Nam	NCGM MHLW
3	Kajio H (NCGM) Anh NQ (BMH) Lien DTK (BMH)	Bach Mai Hospital (BMH)	Impact of a life style intervention in incident and prevalence of overweight and obesity among secondary school children in Hanoi	NCGM MHLW
4	Hiroyuki Shichino (NCGM)	National Hue Central Hospital (Hue) Ho Chi Minh Children Hospital 1 Ho Chi Minh Children Hospital 2 Ho Chi Minh Children Hospital 3 National Children Hospital National Cancer Hospital	Support for Strengthening Medical Treatment Ability of the Childhood Cancer in Viet Nam	NCGM MHLW
5	Naoto Keicho (NCGM/JATA) Pham Huu Thuong (HLH)	Hanoi Lung Hospital (HLH) National Lung Hospital (NLH)	Research on tuberculosis in Viet Nam, Research on spreading Beijing-genotype strains of *Mycobacterium tuberculosis*, their drugresistance profiles and possible effects on treatment outcome	J-GRID MEXT
6	Naoto Keicho (NCGM/JATA) Pham Huu Thuong (HLH)	Hanoi Lung Hospital (HLH)	Research on latent tuberculosis infection among healthcare workers in Hanoi, Viet Nam	J-GRID MEXT
7	Shinsaku Sakurada (NCGM/Kansai International Airport) Naoto Keicho (NCGM/JATA) Pham Huu Thuong (HLH)	Hanoi Lung Hospital (HLH)	Research on HIV/tuberculosis (TB) in Hanoi, Viet Nam	J-GRID MEXT

No.	Main Researchers	Affiliation in Viet Nam	Subject	Source of fund
8	Shinsaku Sakurada (NCGM)	Medical Collaboration Center (NCGM) Bach Mai Hospital (BMH)	Study of Plasma granulysin in HIV tuberculosis (TB) co-infection	J-GRID MEXT
9	Fumihiko Hinoshita (NCGM) Do Gia Tuyen (BMH)	Bach Mai Hospital (BMH)	Research on improvement of CKD and dialysis management in Hanoi, Viet Nam Sister Renal Center Program, International Society of Nephrology	NCGM ISN
10	Hiroshi Ohara (NCGM)	National Institute of Malariology, Parasitology and Entomology (NIMPE) Bach Mai Hospital (BMH)	Study on effective use of the surveillance results for drug resistant pathogens in infection control	NCGM
11	Norio Ohmagari (NCGM) Nguyen Quoc Anh (BMH)	Bach Mai Hospital (BMH)	Research on Epidemiology, Diagnosis and Treatment for Healthcare Associated Infection and Antimicrobial Resistant Bacteria in Viet Nam	J-GRID
12	Miyoshi-Akiyama, Tohru (NCGM)	Bach Mai Hospital (BMH) Cho Ray Hospital	Molecular epidemiology of multidrug-resistant Gram-negative pathogens in medical settings in Viet Nam	J-GRID

Research No.1

1.	**Title(in English)**	The cohort study of HIV-1-infected individuals in Northern Viet Nam
2.	**Title(in Japanese)**	ハノイにおける HIV 感染者のコホート研究
3.	**Main researcher**	Shinichi Oka (AIDS Clinical Center, National Center for Global Health and Medicine, Japan)
4.	**Co-Researcher(s)**	Junko Tanuma, Daisuke Mizushima, Ei Kinai, Hiroyuki Gatanaga, Shoko Matsumoto, Mika Sata, Masafumi Takiguchi, Nguyen Thi Huyen, Nguyen Hoai Dung, Tran Van Giang, Nguyen Vu Trung, Nguyen Van Kinh, Vu Thi Tuong Van, Doan Thu Tra, Do Duy Cuong
5.	**Resource of fund**	Japan Agency for Medical Research and Development (AMED)
6.	**Affiliation(s) in Viet Nam**	National Hospital of Tropical Diseases (NHTD) Bach Mai Hospital (BMH)
7.	**Period of the research**	October 2007- March 2020
8.	**Publications**	1. Tanuma J. et al. ***J Int AIDS Soc.*** 2017 Dec;20(4). 2. Matsumoto S, et al. ***Sci Rep.*** 2017 Nov 14;7(1):15489.

9. **Summary:**

In 2007, we established a hospital-based cohort of HIV-infected individuals in Hanoi, Viet Nam under the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) network, which aimed to enhance the research collaboration on HIV between Japan and Viet Nam. We recruited participants in both the National Hospital for Tropical Diseases (NHTD) and Bach Mai Hospital (BMH) in urban Hanoi and 2,198 HIV-infected individuals have joined the cohort by the end of 2016. Data on demographics, clinical status and laboratory data has been prospectively collected every 6 months and it has now become the longest clinical dataset of HIV-infected patients in Northern Viet Nam.

In 2017, two major analyses were conducted in the cohort, one of which was related to comorbidity of lifestyle-related diseases among patients on antiretroviral therapy (ART), and the other investigated the association between social support and depression among patients on ART. In the former study, the prevalence of dyslipidemia (53.5%) was disproportionally higher compared to the other lifestyle diseases (18.7% for hypertension and 4.2% for hyperglycemia), and the use of lopinavir boosted with ritonavir (LPVr) showed a significant association with dyslipidemia. We will conduct further investigations on incidence of cardiovascular diseases caused by dyslipidemia in other future study. In the latter study, we carried out a cross-sectional survey in NHTD and BMH, in which depression was evaluated using Center for Epidemiological Studies Depression (CES-D) and social support was evaluated using the Medical Outcome Study Social Support Survey (MOS-SSS). In this study, we found that depression was prevalent in 26.2% of participants, and that higher score of social support showed significant association with lower depression rate. Although family was primary source of all types of social support, receiving social support (emotional/informational support) not only from family but also from outside of family correlated with a lower proportion of depression. Out finding suggested that expanding social networks between HIV populations and society is a potentially important option for reducing depression in countries with constrained social resources, as in Viet Nam.

Other currently active researches include;

1) prevalence of drug resistance among those failing ART2) incidence of adverse effects against ART.

2) incidence of adverse effects against ART.

	3) HIV/HBV co-infection 4) prevalence of rickets among children born from mothers receiving ART 5) quality of life measurement by the WHOQOL All of these studies will provide key information on the long-term prognosis of HIV-infected individuals in Viet Nam as well as offered a variety of opportunities for young investigators to work with colleagues from a different country in the field of HIV.

Research No.2

1	Title (in English)	Study on the contribution of obesity to diabetes and blood vascular diseases in Viet Nam
2	Title (in Japanese)	ベトナム人における肥満の糖尿病や心血管疾患への関与に関する研究
3	Main researcher	Kajio H (Department of Diabetes, Endocrinology and Metabolism, NCGM, Japan) Pham MT (BMH, Viet Nam) Nguyen KDV (Department of Diabetes and Endocrinology, BMH, Viet Nam)
4	Co-Researcher(s)	Japan: Matsushita Y (NCGM), Tsujimoto T(NCGM) Viet Nam: Do DL (BMH), Nguyen PA (BMH), Thuy PTP (MCC)
5	Resource of fund	1. The Grant of National Center for Global Health and Medicine, NCGM, Japan 2. The Grant of Ministry of Health, Labor and Welfare of Japan
6	Affiliation(s) in Viet Nam	Bach Mai Hospital
7	Period of the research	May 2011-
8	Publications	None
9	Summary:	

Obesity is supposed to contributing to the deterioration of metabolic abnormalities for diabetes and cardiovascular diseases (CVD). Recently, intra-abdominal adipose tissues, that are VATs, have been found to secret bioactive hormones, which partially regulate the functions of insulin-sensitive organs as well as the vascular functions. The amounts of these hormones are largely dependent on the degree of fat accumulation in VAT. The visceral fat area (VFA) determined as cross-sectional image at the umbilical level using CT or MRI was a superior predictor for the clustering of metabolic risk factors. However, the use of CT or MRI is limited because the methods are not simple or cost-effective. CT and MRI are often unsuitable for screening large number of participants. CT has a problem with X-ray exposure. Recently, several apparatus for the direct measurement have been developed to overcome these problems. Some of them are based on the bioelectrical impedance analysis (BIA). The advantages of BIA include its portability and ease of use, relatively low cost, minimal participant participation required, and safety (not for participants with a pacemaker), thus making it attractive for large-scale studies.

The aims of our study are to establish a system based on abdominal BIA by comparing with the result of CT scan, and to identify directly the association of obesity, especially visceral obesity, and diabetes and blood vascular diseases.

We started the recruitment of the participants in 2016. The participants are 300 subjects (150 males, 150 females), who are being recruited mainly in the outpatient and inpatient clinics of the department of endocrinology and metabolism at Bach Mai Hospital.

At the moment, we have recruited almost 100 subjects. And, we have extended the recruitment of the non-diabetic participants to Out-patient Department. we have been enrolling from 3-5 patients there, so the project is now going very quickly comparing to previous time. To have a good management and encourage our researchers, we hold monthly meetings to review project progress now.

Research No.3

1	Title (in English)	Impact of a life style intervention in incident and prevalence of overweight and obesity among secondary school children in Hanoi
2	Title (in Japanese)	ハノイ市の中学生における肥満・過体重に対する生活習慣介入に関する研究
3	Main researcher	Kajio H (Department of Diabetes, Endocrinology and Metabolism, NCGM, Japan) Anh NQ (BMH, Viet Nam) Lien DTK (Department of Diabetes and Endocrinology, BMH, Viet Nam)
4	Co-Researcher(s)	Japan: Matsushita Y (NCGM), Tsujimoto T(NCGM), Hara M (Tokyo Metropolitan Hiroo General Hospital) Viet Nam: Thanh DVT (BMH), Thanh NTT (BMH), Thuy PTP (MCC)
5	Resource of fund	1. The Grant of National Center for Global Health and Medicine, NCGM, Japan 2. The Grant of Ministry of Health, Labor and Welfare of Japan
6	Affiliation(s) in Viet Nam	Bach Mai Hospital
7	Period of the research	Dec 2012-
8	Publications	In Preparation
9	Summary:	

In the developing countries, the increasing prevalence and incidence of overweight and obesity is a serious public health problem induced by the social and economic changes of the countries. The prevalence has increased at an alarming rate also in children. Overweight and obese children are likely to stay obese into adulthood and more likely to develop non-communicable diseases (NCD) like diabetes and cardiovascular diseases at a younger age. We performed the study on the impact of a life style intervention against overweight and obesity among secondary school children in Hanoi from 2012 to 2016.

We recruited 821 children of 6th grade from 4 different schools (Cat Linh school, Nguyen Cong Tru School, Phan Chu Trinh School, Dong Da school), which were randomly selected from 2 urban districts in Hanoi. After allocation of 4 schools into two groups, two schools for the intervention group and the other two schools for the control group, we performed intervention activities for two years. We provided the participants with the pedometers, and the participants in the intervention group were also provided with the scales. As for the behavior intervention, we promoted the participants in the intervention group to continue the activities with self-monitoring, goal setting, and problem solving. The analysis of the results is now being performed.

We obtained the baseline data from 821 children of 4 schools. We found that the prevalence of children with obesity was 32.4% for males and 7.9% for females, respectively, following WHO standard cut-offs. The data demonstrated that family factors, such as education levels and level of overweight/obesity (OW/OB) of father/ mother, are significantly related with the OR of OW/OB for children. Birth weight and sleeping hour per day, more physical activities to lose weight, less food to lose weight and more vegetable to lose weight are also significantly related with the OR of OW/OB for children. We collected the data of the questionnaires from 739 students and 660 parents and the data of the health check from 731 students at the 2-year final surveillance.

We have already reported part of the results at several scientific meetings. However, we are still under analysis because there are so many topics to be analyzed and discussed.

We revealed the high prevalence of overweight or obesity in school children, and several factors influencing on the appearance of overweight or obesity. It is very important to identify the factors related to the behavioral changes of the students and their parents through the intervention introducing the reduction of the prevalence and incident of overweight

	overweight and obesity in the children. We are now analyzing the topics shown below. 1. Eating behavior and childhood overweight 2. The relationship between distorted body image and lifestyle 3. Overweight/obesity and serum adiponectin complexes 4. LDL cholesterol and body mass index 5. The effectiveness of synchronous intervention for overweight/obesity 6.Blood pressure and childhood overweight 7. Family factors and childhood overweight 8. Sleeping duration and childhood overweight 9. Awareness of diet or exercise and childhood overweight These analyses would help us make the strategy for the intervention of NCD.

Research No.4

1	Title (in English)	Support for Strengthening Medical Treatment Ability of the Childhood Cancer in Viet Nam
2	Title (in Japanese)	ベトナムにおける小児がん医療の診療能力強化を目的とした支援
3	Main researcher	Hiroyuki Shichino (National Center For Global Health And Medicine, Japan)
4	Co-Researcher(s)	Noriko Sato, Junko Yamanaka, Hideko Uryu, Mizue Tanaka, Yuri Yoshimoto
5	Resource of fund	International Promotion of Japan's Healthcare Technologies and Services, NCGM Program International Health Research (A27-5) from Ministry of Health Labor and Welfare of Japan
6	Affiliation(s) in Viet Nam	National Hue Central Hospital (Hue), Ho Chi Minh Children Hospital 1(HCM city), Ho Chi Minh Children Hospital 2(HCM city), Ho Chi Minh Children Hospital 3(HCM city), National Children Hospital(Hanoi), National Cancer Hospital(Hanoi),
7	Period of the research	April 2017- March 2018
8	Publications	七野浩之 , 他：ベトナムの小児がん医療に対する国際医療支援悪経緯と概要．映像情報メディカル 49:56-61,2017 松井基浩、七野浩之：ベトナムの小児医療の現状．映像情報メディカル 49：66-70,2017(昨年報告済み)
9	Summary:	

BACKGROUND:

Eighty percent of world childhood cancer patients are children in the developing countries. There are many problems such as misdiagnoses, delay of discoveries, lack of offer for treatment. Actually there was not the grasp of accurate number of the childhood cancer patients in those countries and many childhood cancer patients were supposed to be untreated. There were small numbers of specialists of pediatric cancer.

PURPOSE:

To support for strengthening diagnosis, medical treatment, supportive care abilities of the childhood cancer in the pediatrics, pediatric surgery, and radiological diagnosis and treatment of leading hospitals in Viet Nam.

METHODS:

1. Sending experts who are well-versed in childhood cancer from Japan, to provide training in the field of childhood cancer diagnosis, treatment, supportive care.
2. Accepting healthcare providers from Viet Nam as trainees for studying childhood cancer.
3. Making a new style of consulting system through the internet cloud environment.

RESULTS:

We sent total 17 Japanese experts to Hue central hospital, Ho Chi Minh Children Hospital 1, Ho Chi Minh Children Hospital 2 and National Children Hospital. And accepted 5 pediatric doctors from Hue, Ho Chi Minh City and Hanoi into Japan. And started to use new consulting system in Hue and Ho Chi Minh. In Hue Central Hospital, there increase the number of children with solid tumors. They didn't treat any children at all in 2016 but they treated more than 20 children in 2017. From Ho Chi Minh Children Hospital 1 they sent more than 10 cases of consulting during 2016-2017.

CONCLUSION:

We could support to improve the medical treatment ability of the staff concerned with childhood cancer about such as a diagnosis, treatment, nursing care, supportive care. And also we thought we could increase the number of childhood cancer patients who had been diagnosed and treated step by step. Consulting system would be useful to keep in touch and continuing of study.

National Children Hospital

Ho Chi Minh Children Hospital 2

National Cancer Hospital

Ho Chi Minh Children Hospital 3

Trainees from Viet Nam studied in NCGM

Research No.5

1	Title (in English)	Research on tuberculosis in Viet Nam Research on spreading Beijing-genotype strains of *Mycobacterium tuberculosis*, their drug-resistance profiles and possible effects on treatment outcome
2	Title (in Japanese)	ベトナムにおける結核症に関する研究 結核菌北京型株の蔓延と多剤耐性に関わる研究
3	Main researcher	Naoto Keicho (NCGM/Research Institute of Tuberculosis, JATA) Pham Huu Thuong (Hanoi Lung Hospital)
4	Co-Researcher(s)	Vu Cao Cuong (Hanoi Department of Health) Nguyen Phuong Hoang (Hanoi Lung Hospital) Nguyen Van Hung (National Lung Hospital) Shinji Maeda (Hokkaido Pharmaceutical University School of Pharmacy) Minako Hijikata (NCGM/Research Institute of Tuberculosis, JATA) Nguyen Thi Le Hang (NCGM-BMH Medical Collaboration Center) Shinsaku Sakurada (NCGM/Quarantine Office, Kansai International Airpport)
5	Resource of fund	The Program of Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), MEXT
6	Affiliation(s) in Viet Nam	Hanoi Lung Hospital (HLH), Viet Nam National Lung Hospital (NLH), Viet Nam
7	Period of the research	2015-2020
8	Publications	Wada T, Hijikata M, Maeda S, Hang NTL, Thuong PH, Hoang NP, Hung NV, Keicho N. Complete Genome Sequence of a Mycobacterium tuberculosis Strain Belonging to the East African-Indian Family in the Indo-Oceanic Lineage, Isolated in Hanoi, Viet Nam. Genome Announc. 2017;Jun 15;5(24), pii: e00509-17. doi:10.1128 Wada T, Hijikata M, Maeda S, Hang NTL, Thuong PH, Hoang NP, Hung NV, Keicho N. Complete Genome Sequences of Three Representative Mycobacterium tuberculosis Beijing Family Strains Belonging to Distinct Genotype Clusters in Hanoi, Viet Nam, during 2007 to 2009. Genome Announc. 2017;Jul 6; 5(27), pii: e00510-17. doi:10.1128 Hang NTL, Hijikata M ,Maeda S, PH Thuong, NP Hoang, NV Hung, Matsushita I, Keicho N. Whole genome sequencing analysis of drug resistance- conferring mutations and lineages/ sublineages of Mycobacterium tuberculosis circulating in Hanoi, Viet Nam. The 48th Union World Conference on Lung Health, 2017. Huyen NT, Thuong PH, Hang NTL, Anh PT, Hijikata M, Matsushita I, Keicho N. Characteristics of previously treated tuberculosis patients in Hanoi, Viet Nam. The 32nd Annual Meeting of Japan Association for International Health 2017 (Joint Conference of Global Health 2017)

9	Summary: **OVERALL PURPOSE:** • To strengthen collaborative research work on tuberculosis (TB) between Viet Nam and Japan. • To prevent generation and spread of drug-resistant TB. **OUTPUT:** **A. NCGM-RIT-HLH collaboration** 1. Analysis of Hanoi-TB data containing clinical, genome-epidemiological, immunological and bacteriological information, and specimens. 2. Improvement of diagnosis, monitoring, treatment and prevention of TB and understanding process of TB infection and development. 3. Identification and reduction of risk factors to prevent spread of drug-resistant TB. 4. Identification of possible risk factors to unfavorable anti-TB treatment outcomes. **B. NCGM-RIT-NLH collaboration** 5. Genome epidemiology of Mycobacterium tuberculosis strains in Hanoi. 6. Analysis of drug-resistant Mycobacterium tuberculosis. 7. Analysis of reactivation and re-infection of Mycobacterium tuberculosis after anti-TB treatment.

Research No.6

1	Title (in English)	Research on latent tuberculosis infection among healthcare workers in Hanoi, Viet Nam
2	Title (in Japanese)	ベトナムにおける医療従事者の潜在性結核感染症に関する研究
3	Main researcher	Naoto Keicho (NCGM/Research Institute of Tuberculosis, JATA) Pham Huu Thuong (Hanoi Lung Hospital)
4	Co-Researcher(s)	Vu Cao Cuong (Hanoi Department of Health) Do Bang Tam (Hanoi Lung Hospital) Minako Hijikata (NCGM/Research Institute of Tuberculosis, JATA) Nguyen Thi Le Hang (NCGM-BMH Medical Collaboration Center) Shinsaku Sakurada (NCGM/Quarantine Office, Kansai International Airport)
5	Resource of fund	The Program of Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), MEXT
6	Affiliation(s) in Viet Nam	Hanoi Lung Hospital (HLH), Viet Nam
7	Period of the research	2015-2018
8	Publications	Hijikata M, Matsushita I, Hang NTL, Tam DB, Huan HV, Cuong VC, Thuong PH, Keicho N. Investigations of RNA/miRNA signature as potential biomarkers for tuberculosis. 6th Conference of International Union Against Tuberculosis and Lung Disease Asia Pacific Region; Tokyo, Japan, 2017
9	Summary: **OVERALL PURPOSE:** • To strengthen collaborative research work on tuberculosis (TB) between Viet Nam and Japan. • To study immunity of latent tuberculosis infection for a better prevention of tuberculosis. **OUTPUT:** 1. To understand human immunity of latent tuberculosis infection, and the process of TB infection and development. 2. Identification of risk factors of tuberculosis infection including occupational factors.	

Research No.7

1	Title (in English)	Research on HIV/tuberculosis (TB) in Hanoi, Viet Nam
2	Title (in Japanese)	ベトナムにおける HIV 感染合併結核に関する研究
3	Main researcher	Shinsaku Sakurada (NCGM/ NCGM/Quarantine Office, Kansai International Airport) Naoto Keicho (NCGM/Research Institute of Tuberculosis, JATA) Pham Huu Thuong (Hanoi Lung Hospital)
4	Co-Researcher(s)	Vu Cao Cuong (Hanoi Department of Health) Do Bang Tam (Hanoi Lung Hospital) Minako Hijikata (NCGM/Research Institute of Tuberculosis, JATA) Nguyen Thi Le Hang (NCGM-BMH Medical Collaboration Center)
5	Resource of fund	The Program of Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), MEXT
6	Affiliation(s) in Viet Nam	Hanoi Lung Hospital (HLH), Viet Nam
7	Period of the research	2015-2018
8	Publications	None
9	Summary: **OVERALL PURPOSE:** • To strengthen collaborative research work on tuberculosis (TB) between Viet Nam and Japan. • To study immunity and risk factors of HIV/TB for a better management of tuberculosis and HIV co-infection. **OUTPUT:** 1. To understand the immunity of HIV/TB co-infection. 2. To identify risk factors of HIV/TB co-infection.	

Research No.8

1	Title (in English)	Study of Plasma granulysin in HIV tuberculosis (TB) co-infection
2	Title (in Japanese)	HIV 結核共感染における血漿グラニュリシンの研究
3	Main researcher	Shinsaku Sakurada (Bureau of International Medical Cooperation, NCGM)
4	Co-Researcher(s)	Nguyen Thi Le Hang (MCC) Le Xuan Hai (NIHBT) Pham Huu Thuong (HLH), Do Bang Tam (HLH) Minako Hijikata (JATA-RIT) Naoto Keicho (JATA-RIT)
5	Resource of fund	The Program of Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), MEXT
6	Affiliation(s) in Viet Nam	Medical Collaboration Center-NCGM/Bach Mai Hospital
7	Period of the research	April, 2015-March, 2018
8	Publications	None
9	Summary:	

Granulysin is a saposin family protein released from NK cells, NKT cells, γδ T cells and cytotoxic T cells (CTL). This molecule induces apoptosis of target cells working with perforin and has significant bacteriocidal activity with broad spectrum.

To study possible roles of granulysin in HIV/TB co-infection, we started a clinical study in Hanoi by recruiting healthy donors as a control group and patients with single infection of HIV and TB, and HIV/TB co-infection in 2012. Blood samples were collected only once in healthy donors and the patients with HIV, twice at the introduction and end of anti-TB treatment in the patients with TB, three times, at the introduction of anti-TB treatment and ART, and at the end of anti-TB treatment in the patients with HIV/TB co-infection. The levels of granulysin in plasma were determined using ELISA and the cellular expression and distribution of granulysin in peripheral blood mononuclear cells were analyzed by flow cytometry using anti-granulysin monoclonal antibody. We will analyze the association between plasma granulysin and clinical manifestation in the patients with HIV/TB co-infection. We completed the recruitment of the patients and conducted the determination of plasma granulysin levels in the samples.

We finalized the determination of plasma granulysin levels using the remained samples in 2017. We are preparing a research paper after comprehensive analysis.

We presented partially the results of this study in 87th Experimental Tuberculosis Research Meeting associated with The Japanese Society For Tuberculosis (Tokyo) on 22 March, 2017.

Research No.9

1	Title (in English)	Research on improvement of CKD and dialysis management in Hanoi, Viet Nam Sister Renal Center Program, International Society of Nephrology
2	Title (in Japanese)	ベトナム国ハノイにおける慢性腎臓病管理・透析の調査と質の向上に関する研究 国際腎臓学会 Sister Renal Center Program
3	Main researcher	Fumihiko Hinoshita (MD, Ph.D, Head, Department of Nephrology, NCGM) Do Gia Tuyen (MD, Ph D, Head, Department of Nephro-Urology, Bach Mai Hospital)
4	Co-Researcher(s)	Manami Tada (MD, Department of Nephrology, NCGM) Nguyen Thi Huong (MD, Department of Nephro-Urology, Bach Mai Hospital)
5	Resource of fund	NCGM (26-3) (-March, 2017) Sister Renal Center Program funded by International Society of Nephrology (ISN)
6	Affiliation(s) in Viet Nam	Bach Mai Hospital
7	Period of the research	(ISN) January 2014- December, 2017 (NCGM 26-3) April 2014- March 2017
8	Publications	None
9	Summary:	

OVERALL PURPOSE:

- To establish further collaboration between Dept of Nephrology, NCGM and Dept of Nephro-Urology, BMH under the Sister Renal Center Program officially approved by International Society of Nephrology (ISN)
- To establish a sophisticated means of treating patients with CKD in the preservation period and retard the progression of CKD in the patients in BMH as well as local hospitals in Northern Viet Nam
- To improve management of hemodialysis at BMH and the dialysis facilities in Hanoi

ACTIVITIES:

- Nephrologists from NCGM and Bach Mai Hospital had lectures on “To realize the high-leveled hemodialysis ” and “Blood pressure management in CKD” at BMH.
- Clinical engineers from NCGM had lectures on maintenance and management techniques of hemodialysis, focusing on hemodialysis water quality
- Two nephrologists from BMH had a training at NCGM
- Upgrading from Level C to Level B under the Sister Renal Center Program was officially approved by ISN in January, 2016 according to the good evaluation of the activities between NCGM and BMH. The status of Level B under the Sister Renal Center Program has continued for two years.
- Nephrologists and a clinical engineer from NCGM and BMH made a poster presentations on “Sister renal centers program between NCGN and BMH” and “Technical assistance efforts for dialysis facilities in and around Hanoi, Viet Nam” at World Congress of Nephrology at Mexico City in April, 2017.
- A clinical research of “The clinical characteristics of the newly hemodialyzed patients with CKD at a major hospital of Hanoi, Viet Nam” is under progress at BMH under the Sister Renal Centers Program.

Research No.10

1	Title (in English)	Study on effective use of the surveillance results for drug resistant pathogens in infection control
2	Title (in Japanese)	院内感染対策における耐性菌サーベイランスの活用
3	Main researcher	Hiroshi Ohara (NCGM)
4	Co-Researcher(s)	Vu Huy Nam (Dept. of Planning, National Institute of Malariology, Parasitology and Entomology) Pham Thi Thanh Thuy (Dept. of Infectious Diseases, BMH) Jeevan B. Sherchand (Dept. of Public Health, Institute of Medicine, Tribhuvan University, Nepal) Pokhrel BM, Shrestha RK, Dahal RK, Mishra SK, Kattel HP, Rijal BP (Dept. of Microbiology, Institute of Medicine, Tribhuvan University, Nepal)
5	Resource of fund	Grants of National Center for Global Health and Medicine (27-4)
6	Affiliation(s) in Viet Nam	National Institute of Malariology, Parasitology and Entomology (NIMPE), Bach Mai Hospital
7	Period of the research	October 2014- March 2017
8	Publications	None

9 Summary:

BACKGROUND AND PURPOSE:

Recently drug resistant pathogens have been spreading. This situation is not only causing issues in treatment, but also has been important factors of nosocomial infections. In developing countries these issues are enlarging but in many counties awareness among medical staff is still low and in actual fact the exact condition is not clear.

The researchers have investigated actual conditions of drug resistant pathogens and nosocomial infection control in Viet Nam and Nepal along with providing technical guidance. This study was designed aiming at contributing to making effective control system utilizing these basic information and latest information.

METHODS:

This study started with reviewing the results of the preceding researches and technical guidance (the researchers' researches and others, document reviews) on antimicrobial resistance of bacteria (AMR), drug resistance of malaria parasites and actual situation of nosocomial infection control. Thereafter, we will conduct comparative analysis between the 2 countries, discuss with health authorities on appropriate control measures and summarize as a proposal.

PROGRESS OF THE STUDY:

1. Viet Nam

Latest information on AMR was collected by interview with tertiary hospital staff and document reviews. High resistance rate for antibiotics have been reported (ex. 75% of *Streptococcus pneumoniae* is multi-drug resistant, 71% of *Klebsiella pneumoniae* is penicillin resistant and 92% is erythromycin resistant, high resistant rate in homophiles). As the cause of resistance, inappropriate use along with governance of antibiotics, abuse for livestock, and communication gap between medical and agricultural sectors etc. were suspected.

Latest information on drug resistance of malaria was also collected. During the past 20 years incidence of malaria has remarkably decreased in Viet Nam, however in recent years new issues such as emergence of new endemic areas in Cambodian border, artemisinin resistance malaria and so on, have been reported.

	2. Nepal We summarized the results of surveys on multi-drug resistant bacteria which we have conducted in Nepal up to 2016. These previous studies have revealed the spread of multi-drug resistant bacteria in medical settings, however measures to address these situations have not been taken effectively. Furthermore, as the results of discussions with authorities in Nepal the following issues were pointed out: inappropriate use of antibiotic, ineffective feedback mechanism of the information on bacterial resistance, inappropriate nosocomoial infection control, poor antibiotic stewardship, weak intervention by the government, etc. As the results of the above researches in Viet Nam and Nepal (1, 2) along with discussion with authorities, the following challenges that we should address, were suspected: use of antibiotics and anti-malaria drugs along with their governance, feedback of resistance information to clinical settings, nosocomial infection control system, sales system of antibiotics, use of antibiotics in livestock farming, population movement, etc. Further investigations on these suspected issues are needed. The results of the study in Nepal in comparison with those in Viet Nam was reported in the following paper. Ohara H, Sherchan JB, Pokhrel BM, Sherchand JB. Review of collaboration between Tribhuvan University Institute of Medicine in Nepal and National Center for Global Health and Medicine in Japan on Nosocomial infection control and proposal for improvement. J Inst Med 2017; 39(2): 101-108.

Research No.11

1	Title (in English)	Research on Epidemiology, Diagnosis and Treatment for Healthcare Associated Infection and Antimicrobial Resistant Bacteria in Viet Nam
2	Title (in Japanese)	ベトナム拠点における医療関連感染症及び後期若耐性菌感染症に関する検討
3	Main researcher	Norio Ohmagari MD, MSc, PhD (Director, Disease Control and Prevention Center, NCGM) Nguyen Quoc Anh MD., PhD. (Director, BMH)
4	Co-Researcher(s)	Teruo Kirikae, MD, PhD (NCGM) Tohru Miyoshi-Akiyama, PhD (NCGM) Tatsuya Tada, PhD (NCGM) Kayoko Hayakawa, MD., PhD (NCGM) Nozomi Takeshita, MD., PhD (NCGM) Satoshi Kutsuna, MD., PhD (NCGM) Maki Nagamatsu (NCGM) Mitsuhiro Tsuchiya, MSc (NCGM) Pham Thi Phuong Thuy BA. MPH (NCGM-BMH Medical Coloration Center) Nguyen Gia Binh, MD., PhD (Head of ICU Dept.) Dao Xuan Co (Vice head of ICU Dept.) Truong Thai Phuong MD., PhD (Head of Microbiology Dept., Bach Mai Hospital) Doan Mai Phuong MD., PhD (Microbiology Dept., Bach Mai Hospital) Do Van Thanh (Infectious Dept. and International Dept. Bach Mai Hospital)
5	Resource of fund	Japan Initiative for Global Research Network on Infectious Diseases (Funded from Ministry of Education, Science and Technology, Culture and Sport of Japan)
6	Affiliation(s) in Viet Nam	Bach Mai Hospital
7	Period of the research	April 1, 2012 to March, 2018
8	Publications	1. Miyoshi-Akiyama T, Tada T, Ohmagari N, Viet Hung N, Tharavichitkul P, Pokhrel BM, Gniadkowski M, Shimojima M, Kirikae T. Emergence and Spread of Epidemic Multidrug-Resistant Pseudomonas aeruginosa. Genome Biol Evol. 2017 Dec 1;9(12):3238-3245. doi: 10.1093/gbe/evx243. PubMed PMID: 29202180; PubMed Central PMCID: PMC5726472. 2. Tada T, Nhung PH, Shimada K, Tsuchiya M, Phuong DM, Anh NQ, Ohmagari N, Kirikae T. Emergence of colistin-resistant Escherichia coli clinical isolates harboring mcr-1 in Viet Nam. Int J Infect Dis. 2017 Oct;63:72-73. doi: 10.1016/j.ijid.2017.07.003. Epub 2017 Jul 10. PubMed PMID: 28705756. 3. Tada T, Tsuchiya M, Shimada K, Nga TTT, Thu LTA, Phu TT, Ohmagari N, Kirikae T. Dissemination of Carbapenem-resistant Klebsiella pneumoniae clinical isolates with various combinations of Carbapenemases (KPC-2, NDM-1, NDM-4, and OXA-48) and 16S rRNA Methylases (RmtB and RmtC) in Viet Nam. BMC Infect Dis. 2017 Jul 4;17(1):467. doi: 10.1186/s12879-017-2570-y. PubMed PMID: 28676118; PubMed Central PMCID: PMC5496404.

9	Summary: 1. Genome Biol Evol. 2017 Dec 1;9(12):3238-3245. doi: 10.1093/gbe/evx243. Emergence and Spread of Epidemic Multidrug-Resistant Pseudomonas aeruginosa. Miyoshi-Akiyama T(1), Tada T(2), Ohmagari N(3), Viet Hung N(4), Tharavichitkul P(5), Pokhrel BM(6), Gniadkowski M(7), Shimojima M(8), Kirikae T(2). Pseudomonas aeruginosa (P. aeruginosa) is one of the most common nosocomial pathogens worldwide. Although the emergence of multidrug-resistant (MDR) P. aeruginosa is a critical problem in medical practice, the key features involved in the emergence and spread of MDR P. aeruginosa remain unknown. This study utilized whole genome sequence (WGS) analyses to define the population structure of 185 P. aeruginosa clinical isolates from several countries. Of these 185 isolates, 136 were categorized into sequence type (ST) 235, one of the most common types worldwide. Phylogenetic analysis showed that these isolates fell within seven subclades. Each subclade harbors characteristic drug resistance genes and a characteristic genetic background confined to a geographic location, suggesting that clonal expansion following antibiotic exposure is the driving force in generating the population structure of MDR P. aeruginosa. WGS analyses also showed that the substitution rate was markedly higher in ST235 MDR P. aeruginosa than in other strains. Notably, almost all ST235 isolates harbor the specific type IV secretion system and very few or none harbor the CRISPR/CAS system. These findings may help explain the mechanism underlying the emergence and spread of ST235 P. aeruginosa as the predominant MDR lineage. 2. Int J Infect Dis. 2017 Oct;63:72-73. doi: 10.1016/j.ijid.2017.07.003. Epub 2017 Jul 10. Emergence of colistin-resistant Escherichia coli clinical isolates harboring mcr-1 in Viet Nam. Tada T(1), Nhung PH(2), Shimada K(3), Tsuchiya M(4), Phuong DM(5), Anh NQ(5), Ohmagari N(6), Kirikae T(7). The mcr-1 was first detected on a plasmid in colistin-resistant Escherichia coli from livestock and patients in China. We described here the emergence of colistin-resistant E. coli clinical isolates harboring mcr-1 on the chromosomes in Viet Nam. To our knowledge, this is the first report of hospital-acquired E. coli isolates harboring mcr-1 in a medical setting in Viet Nam. 3. BMC Infect Dis. 2017 Jul 4;17(1):467. doi: 10.1186/s12879-017-2570-y. Dissemination of Carbapenem-resistant Klebsiella pneumoniae clinical isolates with various combinations of Carbapenemases (KPC-2, NDM-1, NDM-4, and OXA-48) and 16S rRNA Methylases (RmtB and RmtC) in Viet Nam. Tada T(1), Tsuchiya M(2), Shimada K(1), Nga TTT(3), Thu LTA(3), Phu TT(3), Ohmagari N(2), Kirikae T(4)(5).
	METHODS: Twenty-seven clinical isolates of carbapenem-resistant Klebsiella pneumoniae with MICs ≥4 mg/L for imipenem or meropenem were obtained from inpatients in a hospital in Viet Nam. Antimicrobial susceptibility tests and whole genome sequencing were performed. Multilocus sequence typing and the presence of drug resistant genes were determined and a maximum-likelihood phylogenetic tree was constructed by SNP alignment of whole genome sequencing data. **RESULTS:** All the isolates harbored one of genes encoding carbapenemases, including KPC-2, NDM-1, NDM-4 and OXA-48. Of the isolates, 13 were resistant to arbekacin with MICs ≥256 mg/L and to amikacin with MICs ≥512 mg/L. These isolates harbored

a gene encoding a 16S rRNA methylase, either RmtB or RmtC. Eighteen and 4 isolates belonged to international clones, ST15 and ST16, respectively. None of the isolates had colistin-resistant factors.

CONCLUSION:

Carbapenem-resistant K. pneumoniae isolates belonged to international clones spread in a medical setting in Viet Nam, and that these isolates harbored genes encoding various combinations of carbapenemases and 16S rRNA methylases. This is the first report of KPC-2, NDM-4 and OXA-48 producers in a medical setting in Viet Nam.

4. Prospective cohort study of epidemiological findings with nosocomial bloodstream infections in the ICU of a critical care medical center in Viet Nam (Bach Mai Hospital in Hanoi)

BACKGROUND:

Nosocomial infections are a challenging issue for medical facilities around the world. The mortality of bloodstream infection (BSI), the leading nosocomial infection, is high. In Viet Nam, however, no sufficient surveillance of nosocomial infections is performed at medical facilities and the epidemiological information on nosocomial BSI is limited.

METHOD:

We conducted a prospective cohort study of patients diagnosed with BSI in the ICU of a critical care medical center in Viet Nam (Bach Mai Hospital in Hanoi) during the period from December 2013 to August 2015.

RESULTS:

During the observation period, 100 patients were diagnosed with BSI. We defined nosocomial infection in case of patients whose blood for culture was collected 48 hours or longer after admission, or those whose blood for culture was collected before 48 hours after admission, but who had other medical exposure, such as to patients with a nosocomial infection. We analyzed 90 cases of patients with BSI associated with a nosocomial infection, but not with contamination. Among these patients, 59 patients were male (66%) with a median age at the time of the diagnosis of 57 years (IQR: 41–72 years); and 53 patients (59%) had such underlying diseases as diabetes, chronic cardiac disease, chronic renal disease, COPD, blood tumor, or solid tumor. The sources of infection were: CRABSI in 27 patients (30%); PLABSI in 2 (2.2%); others in 32 (35.6%); and unknown in 29 (32.2%). The major causative agents were: Candida spp. in 26 patients (29%); Enterococcus spp. in 19 (19%); E. coli in 13 (13%); A. baumanii in 10 (10%); K. pneumoniae in 10 (10%); MRSA in 4 (4%); and MSSA in 3 (3%). The total hospitalization period was 17 (9-23) days. Of 65 patients who were followed up on day 30 after blood culture collection, 31 patients (48%) were alive an 34 (52%) had died. Univariate logistic regression analysis indicated the following risk factors for death: having any underlying disease (odds ratio: 4.3; 95% CI: 1.5-12.8; $p < 0.01$); and having chronic heart disease (odds ratio: 3.4; 95% CI: 1.1-10.5; $p = 0.03$). Multivariate logistic regression analysis with age and gender as covariates indicated: having chronic heart disease (odds ratio: 3.5; 95% CI: 1.0–11.9; $p = 0.05$).

CONCLUSION:

The epidemiology of nosocomial BSI in an ICU in Viet Nam was different from that in developed countries; we hope that it

	will be further researched in the future. 5. Epidemiology and outcomes of ventilator-associated pneumonia in intensive care units in Viet Nam **BACKGROUND:** In recent years, there is a need for responses to nosocomial infections in developing countries. While it is necessary tointroduce more rapid and appropriate treatment and preventive measures, the epidemiological data serving as the basis of such responses are dominated by those obtained in developed countries. **METHOD:** We conducted a prospective cohort study of patients diagnosed with VAP in the ICU of Bach Mai Hospital in Hanoi, Viet Nam, during the period from November 2015 to April 2016. **RESULTS:** During the observation period, 44 patients were diagnosed with VAP. The median age of the patients was 60 years (IQR: 48–68 years). Among these patients, 33 (75%) were male, and 24 (55%) had such underlying diseases as diabetes, chronic heart disease, chronic renal disease, or COPD. Among the identifiable causative agents, the most frequent ones were A. baumanii (11 patients), K. pneumoniae (7 patients), and P. aeruginosa (4 patients). The number of patients who required treatment with colistin based on the results of drug sensitivity tests was 20 (45%). The number of deaths within 7 days after onset was 6 (14%) and that within 30 days was 16 (36%). The median number of days spent in the ICU after onset was 9 days (IQR: 3–17 days). **CONCLUSION:** The study results suggest that both the frequency of VAP due to multiple-drug-resistant bacteria and the mortality rate are high in ICUs in Viet Nam. Further research, including a study of the proper empiric therapy, is considered necessary for improving the prognosis.

Educational activities:

Training Course on Case Management of Tropical Infectious Diseases was held at Ho Chi Minh City, Viet Nam (December 2017).

Research No.12

1	Title (in English)	Molecular epidemiology of multidrug-resistant Gram-negative pathogens in medical settings in Viet Nam
2	Title (in Japanese)	ベトナムの医療施設における多剤耐性グラム陰性菌の分子疫学解析
3	Main researcher	Miyoshi-Akiyama, Tohru (NCGM)
4	Co-Researcher(s)	Norio Ohmagari (NCGM)
5	Resource of fund	J-GRID
6	Affiliation(s) in Viet Nam	Bach Mai Hospital, Cho Ray Hospital
7	Period of the research	2015-2017
8	Publications	1. Tada, T., Tsuchiya, M., Shimada, K., Nga, T.T.T., Thu, L.T.A., Phu, T.T., Ohmagari, N., Kirikae, T. 2017 Jul 4. Dissemination of Carbapenem-resistant Klebsiella pneumoniae clinical isolates with various combinations of Carbapenemases (KPC-2, NDM-1, NDM-4, and OXA-48) and 16S rRNA Methylases (RmtB and RmtC) in Viet Nam. BMC Infect Dis 17, 467. 2. Miyoshi-Akiyama, T., Tada, T., Ohmagari, N., Viet Hung, N., Tharavichitkul, P., Pokhrel, B.M., Gniadkowski, M., Shimojima, M., Kirikae, T. 2017 Dec 1. Emergence and Spread of Epidemic Multidrug-Resistant Pseudomonas aeruginosa. Genome Biol Evol 9, 3238–3245. 3. Tada, T., Nhung, P.H., Shimada, K., Tsuchiya, M., Phuong, D.M., Anh, N.Q., Ohmagari, N., Kirikae, T. 2017 Oct. Emergence of colistin-resistant Escherichia coli clinical isolates harboring mcr-1 in Viet Nam. Int J Infect Dis 63, 72–73.
9	Summary:	

Pseudomonas aeruginosa (P. aeruginosa) is one of the most common nosocomial pathogens worldwide. Although the emergence of multidrug-resistant (MDR) P. aeruginosa is a critical problem in medical practice, the key features involved in the emergence and spread of MDR P. aeruginosa remain unknown. This study utilized whole genome sequence (WGS) analyses to define the population structure of 185 P. aeruginosa clinical isolates from several countries. Of these 185 isolates, 136 were categorized into sequence type (ST) 235, one of the most common types worldwide. Phylogenetic analysis showed that these isolates fell within seven subclades. Each subclade harbors characteristic drug resistance genes and a characteristic genetic background confined to a geographic location, suggesting that clonal expansion following antibiotic exposure is the driving force in generating the population structure of MDR P. aeruginosa. WGS analyses also showed that the substitution rate was markedly higher in ST235 MDR P. aeruginosa than in other strains. Notably, almost all ST235 isolates harbor the specific type IV secretion system and very few or none harbor the CRISPR/CAS system. These findings may help explain the mechanism underlying the emergence and spread of ST235 P. aeruginosa as the predominant MDR lineage.

2. The International Promotion of Japan's Healthcare Technologies and Services in 2017

This program has been commissioned by the Ministry of Health, Labour and Welfare Japan since fiscal 2016. The purpose is to extend Japanese healthcare and services as well as experiences on health systems to the world. Areas of the program include (1) Japanese health technologies, medical devices, and medicines, (2) management of health facilities, (3) health regulation, medical insurance, medical environment management, (4) health information systems, and (5) global health issues such as emerging and re-emerging infectious diseases, an aging society, maternal and child health, nutrition, non-infectious diseases, and disaster response. The program consists of two methods; dispatch of Japanese specialists and acceptance of foreign trainees in Japan.

The five programs were carried out in Viet Nam by NCGM as follows:

- Support for Strengthening Medical Treatment Ability of Childhood Cancer in Developing Country
- The project for Cambodia, Lao PDR, Myanmar and Viet Nam to strengthen of the ability for instruction about nursing clinical training.
- Technical support project of radiation, clinical examination, clinical engineering department in Asia
- Strengthening Management Capability for Quality and Safety in Healthcare
- Strengthening clinical capacity in Viet Nam

3. Other activities, topics

3.1 International Nursing Practicum for Nursing Students at the National College of Nursing, Japan

We conducted a one-week nursing practicum in Viet Nam as part of the elective subject of International Nursing Practicum for fourth-year undergraduate students in collaboration with Hai Duong Medical Technical University (HMTU), Việt Nam.

The International Nursing Practicum is designed to enhance student's' abilities to understand the current situation of nursing and health care practice in developing countries, whereby promoting the development of nursing theory with international perspectives to facilitate international health cooperation in nursing. As a prerequisite, students are required to complete the international nursing theory course.

Students were divided into groups, and each group was assigned several presentation topics to work toward the goals of the practicum. Before departing for Hai Duong, Việt Nam, where the practicum took place, students rehearsed their presentations in English in order to improve the quality of presentation and share

their knowledge among groups in preparation for the practicum.

On the first day of the practicum, students gave their presentations in front of the faculty members and undergraduate students at HMTU and NCNJ. They then visited several institutions in Hanoi city and Hai Duong province, such as a provincial hospital, a district hospital, a specialty hospital, a leprosy village, a social welfare institution, and a community health center.

On the last day of the practicum, each group presented the summary of students' experiences at HMTU. Back in Japan at NCNJ, a poster presentation was held in the entrance hall, which gave students an opportunity to summarize what they had learned through the practicum in both Japan and Việt Nam, as well as to inform other junior students and faculty members of their valuable experiences.

Student evaluation revealed that most students wished to contribute what they had learned to nursing activities in Japan and promotion of international health cooperation.

3.2 Development of Fundamental International Network for Multi-regional Clinical Trial

Tatsuo Iiyama, Sr. Manager, International Trials Section, Center for Clinical Sciences, NCGM

The ongoing global health transition is characterized by a shift in disease burden with both communicable to non-communicable diseases (NCD). Because of several unfavorable reasons for private companies, there are neglected patients without opportunities of effective examinations and treatments.

Japan has developed academic surroundings to facilitate "Investigator-initiated Clinical Trials" following ICH regulations, aiming for reinforcement of medical innovation, and therapeutic optimization by producing reliable clinical evidence.

We founded an Academic CRO in 2017. We also started to develop new relationships about Multi-Regional Clinical Trials (MRCTs) with medical institutions in Viet Nam, including Bach Mai Hospital, Cho Ray Hospital, National Institute of Infectious and Tropical Diseases (NIITD), National Hospital for Tropical Diseases (NHTD) and the National Hospital of Pediatrics (NHP).

Because 2017 is the beginning period to make a new international framework, we firstly discuss and recognize the overall concept of International Investigator-initiated trial. The discussions include comprehensive themes such as background, purpose, role, regulations, management systems, methodology, scientific design, statistics, data management, ethics, risks, indemnification, quality management, organization, economic affairs, procurements etc.

For more profound understandings with each other, we held or participated informative programs with stakeholders following,

<Sakura Science Program (Youth exchanging program) >

Location: Tokyo
Period: (1) 22nd-30th Nov 2016 (2)16th -24th Jan 2018
Contents: Clinical Trial Methodology, Topics of Cutting-edge Medical Innovation, Infection
Participants: 8 clinicians and researchers from 4 countries (IDN, PHL, THA, VNM)
URL: https://ssp.jst.go.jp/report2016/k_vol113.html

<PMDA-ATC Pharmaceuticals Seminar 2017 in Hanoi, Viet Nam>

Location: Hanoi
Period: 3rd-4th Oct 2017
Contents: Regulatory Issues in MRCTs, Tropical Medicine, Infection
Participants: 30 regulators working for Drug Administration of Viet Nam (DAV)
URL: https://www.pmda.go.jp/english/symposia/0119.html

<Oda-memorial international symposium>

Location: NCGM, Tokyo
Period: 18th Oct 2017
Contents: Conditions of MRCTs in Southeast Asia, USA, and Japan
http://www.ncgm.go.jp/pressrelease/2017/pdf/20170907.pdf
Participants: lecturers from IDN, PHL, THA, VNM, USA, and Japan. 136 Audiences.
URL(leaflet): http://www.ncgm.go.jp/pressrelease/2017/pdf/20170907.pdf

For efficient collaborative activities among counterparts in Viet Nam and Japan, A new Vietnamese staff will join our organization as the project manager in March 2018. After developing basic consensus and functional condition for ICH-following MRCTs, new protocols are going to be launched onward.

III. Reference

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RESEARCH ARTICLE

Long-term viral suppression and immune recovery during first-line antiretroviral therapy: a study of an HIV-infected adult cohort in Hanoi, Vietnam

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Abstract

Introduction: Achieving viral suppression is key in the global strategy to end the HIV epidemic. However, the levels of viral suppression have yet to be described in many resource-limited settings.

Methods: We investigated the time to virologic failure (VF; defined as a viral load of ≥1000 copies/ml) and changes in CD4 counts since starting antiretroviral therapy (ART) in a cohort of HIV-infected adults in Hanoi, Vietnam. Factors related to the time to VF and impaired early immune recovery (defined as not attaining an increase in 100 cells/mm^3 in CD4 counts at 24 months) were further analysed.

Results: From 1806 participants, 225 were identified as having VF at a median of 50 months of first-line ART. The viral suppression rate at 12 months was 95.5% and survival without VF was maintained above 90% until 42 months. An increase in CD4 counts from the baseline was greater in groups with lower baseline CD4 counts. A younger age (multivariate hazard ratio (HR) 0.75, vs. <30), hepatitis C (HCV)-antibody positivity (HR 1.43), and stavudine (d4T)-containing regimens (HR 1.4, vs. zidovudine (AZT)) were associated with earlier VF. Factors associated with impaired early immune recovery included the male sex (odds ratio (OR) 1.78), HCV-antibody positivity (OR 1.72), d4T-based regimens (OR 0.51, vs. AZT), and nevirapine-based regimens (OR 0.53, vs. efavirenz) after controlling for baseline CD4 counts.

Conclusion: Durable high-rate viral suppression was observed in the cohort of patients on first-line ART in Vietnam. Our results highlight the need to increase adherence support among injection drug users and HCV co-infected patients.

Keywords: HIV; viral load; injection drug use; hepatitis C; antiretroviral therapy; Vietnam

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1 | INTRODUCTION

The increased accessibility to antiretroviral therapy (ART) has dramatically reduced the mortality and morbidity of HIV-infected individuals globally. Recent studies have also demonstrated the significant benefits of early ART initiation and preventive ART use [1,2], which had accelerated ART promotion. In 2014, the Joint United Nations Programme on HIV/AIDS (UNAIDS) set the "90-90-90" goal, which aims to diagnose 90% of all HIV-infected individuals, provide ART to 90% of diagnosed individuals, and achieve undetectable HIV RNA levels in 90% of individuals receiving ART by 2020 [3]. According to the 90-90-90 strategy, the plasma HIV viral load (VL) test is considered an essential tool to evaluate the progress of the third goal. Routine VL monitoring has been the gold standard to detect treatment failure since the mid-1990s in resource-rich countries, and the World Health Organization (WHO) recommends VL monitoring after six months of ART and then every 12 months whenever possible [2,4,5]. However, VL monitoring is still unavailable for many ART programmes in resource-limited countries, given the procedure's cost and complexity, which has limited information regarding viral suppression rates in these regions.

VL monitoring identifies individuals who need additional support to adhere to their ART regimen or when to switch from the current regimen to salvage ART, which can facilitate effective resource allocation and investment. Vietnam has recently expanded ART programme with a coverage rate of 68% by the end of 2013 [6], although most patient groups have not experienced the benefits of VL monitoring. The HIV epidemic in Vietnam is strongly associated with injection drug use (IDU; 45%) and transmission to their sexual partners [6], and the overall virologic outcome of ART may be influenced by the social and epidemiological characteristics of these high-risk groups. Several previous studies, including three Vietnamese studies, have revealed suboptimal adherence to ART

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among drug users [7–11], although those studies only examined small target populations during short study periods [11–16]. As ART requires lifelong optimal adherence to experience its full benefits, it would be preferable to examine long-term virologic outcomes that are related to treatment failure, such as death and loss to follow-up. Therefore, we aimed to determine the time to virologic failure (VF), which was defined as having an HIV VL of ≥1000 copies/ml during first-line ART [4], as well as longitudinal immune recovery, and their related risk.

2 | METHODS

2.1 | Study setting and population

Analyses were conducted with a longitudinal dataset from the Hanoi HIV Cohort Study [17], which was established in October 2007 in two large hospitals in urban Hanoi, Vietnam, namely National Hospital of Tropical Diseases (NHTD) and Bach Mai Hospital (BMH); both of these hospitals are teaching and referral hospitals and provide free ART programmes. HIV-infected individuals aged 18 years or above were consecutively enrolled from October 2007 to April 2013 and followed up until April 2015.

For this study, we enrolled patients who were on ART since October 2007 to March 2012 and extended the eligibility to untreated patients in April 2012. Retrospective and prospective data were obtained at the time of enrolment and every six months during ART and included all ART histories and reasons for ART change. Free VL testing had been provided as part of the programme, using the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test system (Roche Diagnostics Ltd., Rotkruez, Switzerland). The VL testing was performed every six months until an undetectable VL was observed, and was subsequently performed at least annually thereafter. Genotypic drug resistance testing was performed for participants with a VL of >1000 copies/ml. The test results were returned to the treating physicians, and the ART was switched to a salvage regimen for participants with a VL of >1000 copies/ml with any drug resistance mutation or two consecutive VLs of >1000 copies/ml. Patients who had no VL test results during first-line ART, were on ART for less than 7 days, or had started with mono/dual therapy or regimens that contained protease inhibitor or nucleos(t)ide reverse transcriptase inhibitor (NRTI) combinations other than zidovudine (AZT), stavudine (d4T), or tenofovir (TDF) plus lamivudine (3TC) or emtricitabine (FTC), were excluded from the analyses. Diagnosis, prophylaxis, and treatment of opportunistic infections and indication and selection of antiretroviral drugs were decided based on the Vietnamese national guidelines [18–20], which were updated twice during the study period according to changes in the WHO guidelines [4,21,22]. The CD4 count for ART indication was increased from 200 to 250 cells/mm^3 in 2009 [19] and to 350 cells/mm^3 in 2011 [20]. AZT and d4T were replaced with TDF as the preferred first-line drug in 2011 [20].

The study protocol was approved by the ethics committee in the Vietnamese Ministry of Health (No: 1666/QD-BYT) and the institutional ethical review boards in BMH, NHTD, and the National Center for Global Health and Medicine in Tokyo, Japan (NCGM-G-001074-01). All study participants provided written informed consent before study enrolment.

2.2 | Statistical analysis

The time from ART initiation to treatment failure was analysed based on VF or a combined clinical endpoint, which was defined as the first episode of death, or change in ART drug due to lack of efficacy or VF after ≥6 months of first-line ART. First-line ART was defined as the ART prescribed for the first time in the patient's life and was considered to be continued until the last clinic visit or a change in ART due to lack of efficacy. Loss to follow-up was defined as cases of patients whose data for 12 months before the last day of data collection in the database were not found, and the date of the last clinic visit was used as the date of loss to follow-up. For both endpoints, the patients were censored at the end of the observation period or when they were transferred to another clinic. In addition, patients were censored in the VF analysis in cases of death, loss to follow-up, or change in the ART. The baseline CD4 cell count at ART initiation was defined as the closest CD4 measurement prior to and within three months of ART initiation. The mean CD4 count and mean CD4 count change trajectories were estimated using linear regression with the outcome as a function of time (in years). For the latter, to ensure flexibility in the estimated shape of the trajectories, we used a natural cubic spline with three knots at six months, three years, and six years [23,24].

We analysed the risk factors for early VF using the Cox proportional hazards model, and a logistic regression model was used to analyse the risk factors for failed early immune recovery, which was defined as not achieving an increase in CD4 counts of 100 cells/mm^3 at 24 months. Both analyses included the following covariates: age at ART initiation, sex at birth, AIDS history before ART, baseline CD4 count, previous IDU, a positive hepatitis B virus surface antigen (HBs antigen) result, a positive anti-hepatitis C virus antibody (HCV-antibody) result, and antiretroviral categories of NRTIs (AZT, d4T, or TDF) and non-nucleoside reverse-transcriptase inhibitors (nevirapine (NVP) or efavirenz (EFV)). Variables that were significant for the univariate analysis ($p < 0.10$) were chosen for the multivariate analysis and considered statistically significant at $p < 0.05$ in the final model. The chi-square test was used to evaluate the association between IDU history and HCV-antibody positivity. All statistical analyses were performed using STATA software (version 12; StataCorp LP, College Station, TX, USA) and R Statistical Software (version 3.2.0; Foundation for Statistical Computing, Vienna, Austria) [23].

3 | RESULTS

3.1 | Baseline characteristics

Between October 2007 and May 2013, 2156 HIV-infected individuals were enrolled in the Hanoi HIV Cohort Study. This study excluded 350 patients, including 185 patients who never underwent ART, 48 patients who underwent ART for less than six months, 96 patients whose initial ART did not match the study criteria, 15 patients without VL results during their first-line ART, five patients whose ART start date was unknown, and one patient who was ≤18 years old at the start

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of ART. The remaining 1806 patients provided 7752 person-years of follow-up and were assigned to the survival analysis with virologic outcome categories. Data of 1441 patients with available baseline CD4 counts were used for CD4 count and CD4 count change trajectories, and 1013 of the 1441 patients who had CD4 results at 24 months were assigned to the immune recovery analysis category. The characteristics of study participants are summarized in Table 1. Overall, 64% of the participants were men and the median age was 31 years (range, 18–75). Thirty-two percent declared previous IDU, and 45% had HCV co-infection, which strongly indicates exposure to sharing multiple needles. The median baseline CD4 count was greater after 2011, compared to before 2011, which was related to the updated ART indication in the national guidelines (before 2011 median 100 cells/mm^3 (ranged 1–671) vs. after 2,011,158 cells/mm^3 (ranged 1–693)).

3.2 | Time to VF and combined clinical endpoints

In a median time of 50 months of first-line ART, 225 individuals (12.4%) experienced VF, which corresponded to an incidence of 2.9/100 person-years. Among these 225 individuals, 47 individuals were identified as having a VL ≥1000 copies/ml within 12 months and 18 individuals subsequently achieved an undetectable VL. The combined clinical endpoint was detected for 311 individuals, which corresponded to an incidence of 4.0 cases/100 person-years. These cases included 225 cases of VF, 36 deaths, and 50 cases of loss to follow-up. By dividing the group that received a VL test by the group with VLs below each threshold, we observed that the proportion of viral suppression at 12 months was calculated to be 95.5% (1329/1391) for a threshold of 1000, 92.9% (1292/1391) for a threshold of 200, and 80% (1112/1391) for a threshold of 1000. Figure 1 illustrates the survival curves with neither VF nor the combined clinical failure. The probability of survival without VF was maintained above 90% until 42 months of first-line ART, with probabilities of 86% at five years and 78% at ten years. The probability of survival without the combined clinical endpoint was 80% at five years and 70% at ten years.

In the univariate analyses, earlier VF was associated with IDU, HCV-antibody positivity, and d4T use were associated with an earlier VF. However, protective effects were observed for age ≥30 years, HBs antigen positivity, a baseline CD4 count of ≥200 cells/mm^3, and an EFV-based regimen showed significant protective effects (Table 2). Of these factors, we found HCV-antibody positivity was strongly associated with IDU history ($p < 0.001$) with 83% concordance, which may have affected the result of the multivariate analysis when both factors were integrated into the same multivariate model. As individuals with IDU may have been reluctant to disclose their IDU, we considered the presence of antibodies to HCV a better indicator of sharing needs, compared to a self-reported history of IDU. Thus, we developed two multivariate models, with one that included all significant factors in the univariate model (Model 1-1) and another that included all significant factors except IDU (Model 1-2). Model 1-1 revealed a significant result for age of >30 years, while Model 1-2 also revealed a significant result for antibodies to HCV.

In the univariate analyses, the combined clinical endpoint was associated with the male sex, IDU, HCV-antibody positivity, and d4T. However, protective effects were observed for age of >30 years and a baseline CD4 count of ≥200 cells/mm^3. We also developed two multivariate models for the combined clinical endpoint (Model 2-1 and Model 2-2) and both models provided similar results, with shorter survival being significantly predicted by antibodies to HCV and d4T use.

Table 1. Characteristics of study participants

Number of participants	Total 1806	Immune recovery study 1013
Median age at starting ART	31 (18–75)	34 (18–75)
<30 years old	668 (37)	352 (35)
30 to 39 years old	845 (47)	495 (49)
≥40 years old	293 (16)	166 (16)
Male sex	1156 (64)	642 (63)
HIV risk factors (multiple possible)		
Sexual contact	1334 (74)	755 (75)
Injection drug use, n (%)	583 (32)	308 (30)
Other/unknown	126 (7)	74 (7.3)
HBs antigen		
Positive	242 (14)	135 (14)
Negative	1507 (86)	863 (86)
Missing	57	15
Anti-HCV antibody		
Positive	737 (45)	385 (42)
Negative	897 (55)	527 (58)
Missing	172	101
AIDS before ART	307 (17)	202 (20)
Median baseline CD4 count (/mm^3)	101 (1 to 693)	81 (1 to 693)
<100	712 (39)	556 (55)
100 to 199	377 (21)	266 (26)
≥200	352 (19)	191 (19)
Missing	365	-
Median time on ART, months	50 (6–152)	53.5 (7–119)
Year of ART initiation		
≤2008	514 (29)	203 (20)
2009 to 2010	518 (28)	384 (38)
≥2011	774 (43)	426 (42)
NRTI		
AZT + 3TC or FTC	832 (46)	523 (52)
d4T + 3TC or FTC	608 (34)	349 (34)
TDF + 3TC or FTC	366 (20)	141 (14)
NNRTI		
NVP	958 (53)	556 (55)
EFV	848 (47)	457 (45)

Data are reported as number (%) or median (range). ART, antiretroviral therapy; HBs antigen, hepatitis B surface antigen; HCV, hepatitis C virus; NRTI, nucleos(t)ide reverse transcriptase inhibitors; AZT, zidovudine; d4T, stavudine; TDF, tenofovir; 3TC, lamivudine; FTC, emtricitabine; NNRTI, non-nucleoside reverse-transcriptase inhibitors; NVP, nevirapine; EFV, efavirenz.

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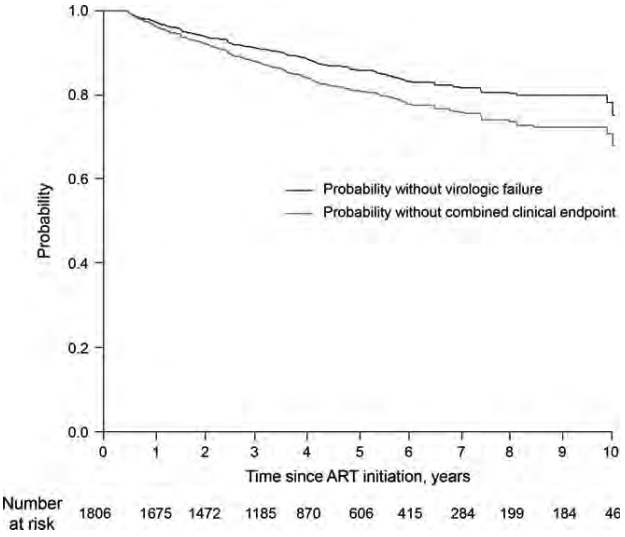

Figure 1. Time to virologic failure (VF) or the combined clinical endpoint during the first-line ART. The black line indicates the probability of not achieving virologic endpoint and the red line indicates the probability of not achieving the combined clinical endpoint. The virologic endpoint was defined as the first episode of VF, (a viral load of ≥1000 copies/ml after starting ART. The combined clinical endpoint was defined as the first episode of either VF, death, loss to follow-up or switching to a salvage regimen. ART, antiretroviral therapy.

3.3 | Immune recovery during first-line ART

Figure 2 shows that the absolute CD4 counts in 1441 patients increased over time regardless of their baseline CD4 count categories <100 cells/mm^3, 100 to 199 cells/mm^3, or ≥200 cells/mm^3. Although 95% confidence intervals are not shown in Figure 2, the three baseline category-specific intervals crossed at approximately 6.5 years. The mean change in CD4 count was 155 cells/mm^3 in the first year and 255 cells/mm^3 at 24 months (Figure 2b) with 167 of 1013 (16.5%) individuals failing to achieve early immune recovery.

In the univariate model, impaired early immune recovery was associated with the male sex, IDU, and HCV-antibody positivity. Protective effects were observed for d4T and EFV (Table 3) other than baseline CD4 categories. Given the strong association between HCV-antibody positivity and IDU mentioned above, we also developed models for impaired early immune recovery that did and did not incorporate IDU history (Model 3-1 and Model 3-2). Both models provided similar results compared to the univariate models, although IDU was a significant factor in Model 3-1.

4 | DISCUSSION

This study is the first to address the long-term virologic efficacy of first-line ART in Vietnam, and to explore factors that were related to VF and early immune recovery. The results revealed high-level durable viral suppression and favourable CD4+ recovery. A younger age and HCV co-infection were associated with early VF and immune recovery at 24 months was more likely to be impaired among men and individuals with HCV co-infection. The currently recommended EFV-containing regimen was associated with a lower VF rate and higher early immune recovery rate, compared to the NVP-containing regimen, and d4T was associated with a higher VF rate than AZT.

The viral suppression rate at 12 months in this study (95.5%) was comparable to that of a previous study (93.5%) [15] and was higher than that of previous small studies in Vietnam (70% to 78%) [12–16]. Furthermore, the 80% suppression at 12 months based on a threshold VL of 50 copies/ml was comparable to that observed in resource-rich countries [25]. Consistently favourable outcomes were even observed for the combined clinical endpoint, which accounted for death/loss to follow-up and eliminated bias caused by early censoring of these outcomes [25]. Similarly, our previous studies that revealed a high retention rate in care and low mortality in this cohort [17,26]. The excellent virologic outcome in our study strongly supports the achievability of Vietnam's goal to end AIDS by 2030 [6] in addition to the UNAIDS 90-90-90 goal [3].

We found that VF was associated with age of <30 years and antibodies to HCV, although self-reported IDU was not significantly associated with VF. Given the legal consequences of IDU in Vietnam and the strong link between IDU and HCV acquisition, it is highly probable that IDU was underreported by the patients and should still be considered a risk for VF. Previous studies have also reported that younger age and IDU were associated with suboptimal adherence to ART [15,26]. Our results indicated that these populations need more support for ART adherence. In addition, we unexpectedly found that individuals with HBs antigen were less likely to develop VF, which may be related to the management of hepatitis in the national ART guidelines [19,20,28], which state that cessation of an anti-HBV drug in an ART regimen may have motivated physicians and patients not to miss a dose. Furthermore, a lower baseline CD4 count (<200 cells/mm^3) was associated with a higher risk of VF in the univariate model, which has also been observed in previous studies [15]. A lower baseline CD4 count might be the result of a combination of social and clinical factors. For example, a late diagnosis of HIV, behaviour wherein individuals reduced likelihood of seeking care, barriers to accessing healthcare, and lack of appropriate knowledge among healthcare providers on when to start ART are plausible risk factors for VF among those with lower baseline CD4 counts. However, having AIDS prior to ART was not associated with VF, although most AIDS patients in this cohort had tuberculosis (TB) and the mortality of HIV/TB co-infection was relatively low, compared to that in other resource-limited countries [17]. Since TB treatment also requires strict adherence, the additional involvement of a TB specialist may increase the likelihood that the patient adheres to the ART.

This study revealed that d4T use was associated with a higher probability of VF than AZT, even though previous clinical trials had revealed minimal difference in virologic efficacy between d4T and AZT [29]. However, EFV use was associated with a lower probability of VF compared to NVP in the univariate model, which was further supported by data for both in vitro potency [30] and from previous clinical trials [31–33].

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Table 2. Factors associated with time to treatment failure during the first-line ART

	VF[a]			Combined clinical endpoint[b]		
		Multivariate, HR (95% CI), *p*			Multivariate, HR (95% CI), *p*	
n = 1806	Univariate, HR (95% CI), *p*	Model 1-1	Model 1-2	Univariate, HR (95% CI), *p*	Model 2-1	Model 2-2
Age at starting ART (years)						
<30	1.00	1.00	1.00	1.00	1.00	1.00
30 to 39	**0.63 (0.47 to 0.83)**, 0.001	**0.69 (0.49 to 0.99)**, 0.04	**0.69 (0.49 to 0.99)**, 0.04	**0.73 (0.58 to 0.92)**, 0.007	0.77 (0.56 to 1.04), 0.08	0.77 (0.56 to 1.04), 0.09
≥40	**0.64 (0.42 to 0.98)**, 0.039	0.71 (0.41 to 1.22), 0.21	0.70 (0.41 to 1.21), 0.20	1.09 (0.80 to 1.48), 0.60	1.02 (0.67 to 1.58), 0.94	1.02 (0.66 to 1.57), 0.94
Male sex	1.11 (0.84 to 1.47), 0.45	0.79 (0.51 to 1.24), 0.31	0.83 (0.55 to 1.25), 0.38	**1.41 (1.10 to 1.81)**, 0.007	0.85 (0.57 to 1.25), 0.40	0.88 (0.61 to 1.27), 0.50
Injection drug use	**1.44 (1.11 to 1.88)**, 0.006	1.15 (0.71 to 1.87), 0.57		**1.62 (1.29 to 2.02)**, <0.001	1.15 (0.76 to 1.73), 0.51	
HBs antigen	**0.64 (0.40 to 1.01)**, 0.058	**0.58 (0.31 to 1.05)**, 0.07	**0.58 (0.31 to 1.06)**, 0.07	0.73 (0.50 to 1.05), 0.09	0.78 (0.49 to 1.20), 0.26	0.78 (0.50 to 1.21), 0.28
Anti-HCV antibodies	**1.42 (1.09 to 1.87)**, 0.01	**1.53 (0.95 to 2.46)**, 0.08	**1.64 (1.06 to 2.12)**, 0.015	**1.60 (1.27 to 2.02)**, <0.001	**1.62 (1.09 to 2.43)**, 0.02	**1.74 (1.24 to 2.44)**, 0.001
AIDS before ART	1.00 (0.70 to 1.43), 0.99			1.08 (0.81 to 1.46), 0.60		
Baseline CD4 count (/mm^3)						
<100	1.00			1.00	1.00	1.00
100–199	1.03 (0.73 to 1.45), 0.87	0.99 (0.69 to 1.42), 0.95	0.99 (0.69 to 1.43), 0.97	0.90 (0.67 to 1.22), 0.52	0.91 (0.66 to 1.26), 0.58	0.92 (0.66 to 1.27), 0.60
≥200	**0.56 (0.34 to 0.93)**, 0.03	0.63 (0.37 to 1.07), 0.09	0.63 (0.37 to 1.07), 0.09	**0.58 (0.38 to 0.88)**, 0.011	0.74 (0.47 to 1.15), 0.18	0.72 (0.47 to 1.15), 0.18
NRTI						
AZT + 3TC or FTC	1.00	1.00	1.00	1.00	1.00	1.00
d4T + 3TC or FTC	**1.35 (1.03 to 1.78)**, 0.03	**1.39 (1.01 to 1.97)**, 0.06	**1.40 (0.99 to 1.97)**, 0.06	**1.38 (1.09 to 1.74)**, 0.007	**1.51 (1.12 to 2.03)**, 0.007	**1.51 (1.12 to 2.04)**, 0.006
TDF + 3TC or FTC	0.80 (0.48 to 1.34), 0.40	1.16 (0.62 to 2.18), 0.65	1.15 (0.61 to 2.16), 0.66	0.87 (0.57 to 1.34), 0.63	0.94 (0.55 to 1.58), 0.80	0.93 (0.55 to 1.57), 0.78
NNRTI						
NVP	1.00	1.00	1.00	1.00		
EFV	**0.72 (0.55 to 0.96)**, 0.025	0.77 (0.52 to 1.14), 0.20	0.77 (0.52 to 1.14), 0.19	1.00 (0.80 to 1.26), 0.99		

HR, hazard ratio; CI, confidence interval; ART, antiretroviral therapy; HBs antigen, hepatitis B surface antigen; HCV, hepatitis C virus; NRTI, nucleos(t)ide reverse transcriptase inhibitors; AZT, zidovudine; d4T, stavudine; TDF, tenofovir; 3TC, lamivudine; FTC, emtricitabine; NNRTI, non-nucleoside reverse-transcriptase inhibitors; NVP, nevirapine; EFV, efavirenz VF, virologic failure.
[a]VF was defined as the first episode of a viral load of ≥1000 copies/ml after the initiation of ART.
[b]The combined clinical endpoint was defined as the first episode of either VF, death, loss to follow-up, or switching to a salvage regimen. Factors with a $p < 0.1$ in the univariate model were included in the multivariate models. Confidence intervals that did not overlap the null value of (HR = 1) are shown in bold.

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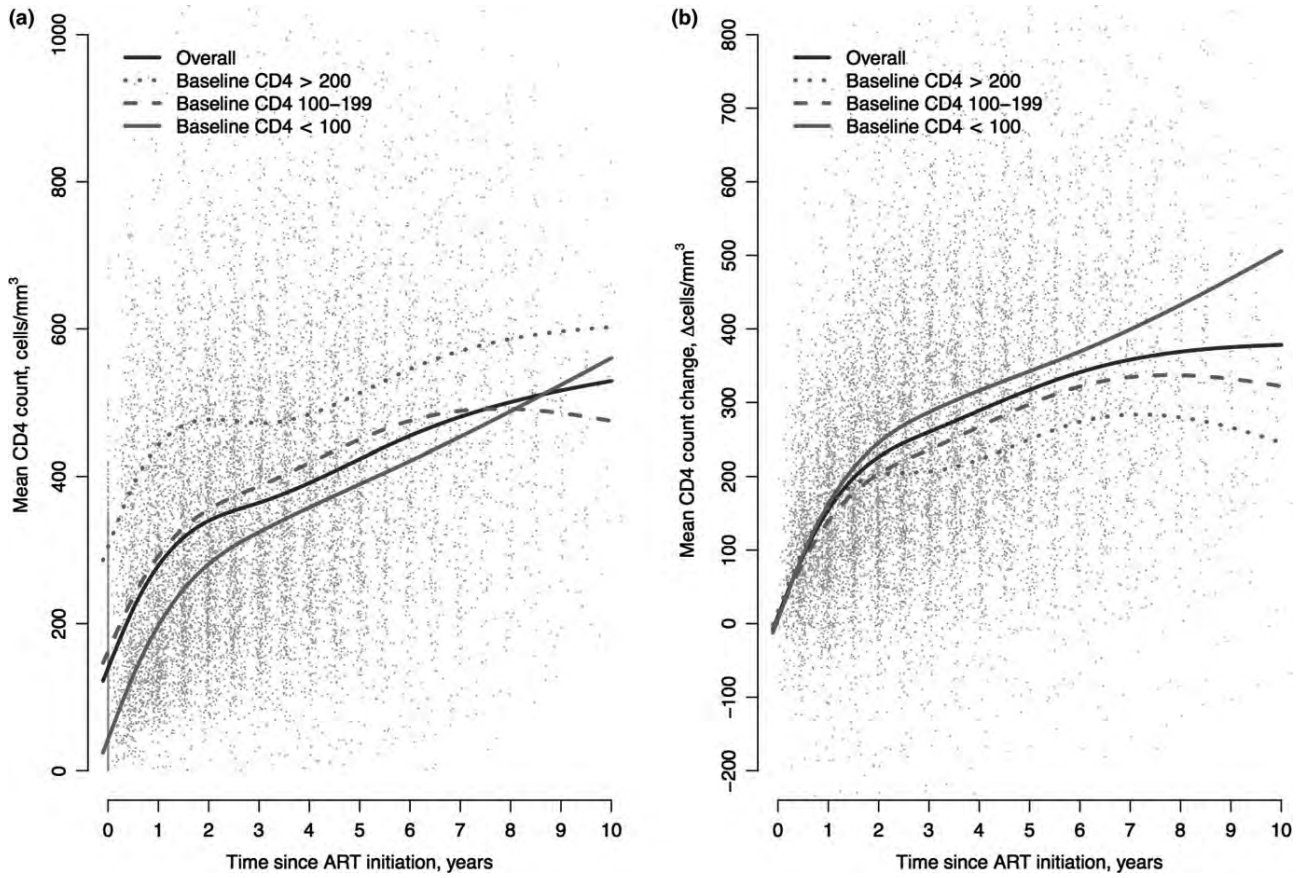

Figure 2. CD4 count trajectory during the first-line ART (a) The mean absolute CD4 counts during the first-line ART, (b) The mean CD4 count change from baseline during the first-line ART. ART, antiretroviral therapy.

One possible reason for the inferiority of d4T in this study might be suboptimal adherence, which could be caused by side effects that are related to mitochondrial toxicity. Nevertheless, it is difficult to assess the actual effects of a single antiretroviral agent on virologic outcome in real-world settings. The Vietnamese national guideline previously recommended d4T/3TC/NVP for first-line ART regimens [18], although the recommendations were changed to AZT and EFV in 2009 [19] and then the combination of TDF/EFV plus 3TC [20] in 2011. Moreover, d4T has been very rapidly phased out of use since 2011 based on global concerns regarding mitochondrial toxicity [22]. Thus, d4T was more frequently used with NVP than EFV, while TDF, which has a high genetic barrier [34], was exclusively used with EFV. Therefore, although it is difficult to evaluate the efficacy of every specific regimen, our data may suggest that the current recommendation of TDF/3TC or FTC/EFV is more beneficial than d4T- or NVP-containing regimens.

We examined the longitudinal immune recovery that occurs during first-line ART and explored factors related to early immune recovery. A relatively large change in CD4 counts was observed early during the ART, which agrees with the findings of other studies [35,36]. However, the rate of increase in CD4 counts was slightly faster in the groups with lower baseline CD4 counts. Our analyses revealed that it took approximately 6.5 years for the mean CD4 count trajectories to become indistinguishable based on overlapping the 95% confidence, which supports the recent global trend toward early ART initiation [2,27].

We found that male sex, IDU, and HCV-antibody positivity were related to impaired early immune recovery, which agrees with the findings of previous studies [37,38]. In this context, chronic immune activation by HCV co-infection may lead to further immune dysfunction [38] and liver cirrhosis may contribute to low leukocyte counts. This relationship is particularly important in Vietnam, where HCV co-infection is distinctly frequent among male HIV-infected individuals. In addition, AZT use was linked to impaired early immune recovery, while EFV use was associated with a more favourable early immune recovery. The mechanisms whereby AZT-containing regimens cause impaired immune recovery remain unclear, although the relationship is commonly accepted and generally explained by AZT-related bone marrow suppression. Although the immune recovery in EFV-based regimens h is generally considered similar to that in NVP-based regimens [39,40], there remains controversy regarding which drug provides the better CD4 response h. Nevertheless, EFV is preferred for Vietnamese patients who are co-infected with HCV than NVP based on concerns regarding the hepatic toxicity of NVP [19,20,27], which may eventually provide an immune recovery benefit among HCV co-infected patients.

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Table 3. Risk factors for impaired early immune recovery after 24 months of the first-line ART

		Multivariate, OR (95% CI), *p*	
n = 1013	**Univariate, OR (95% CI), *p***	**Model 3-1**	**Model 3-2**
Age at starting ART (years old)			
<30	1.00	1.00	1.00
30 to 39	1.21 (0.83 to 1.76), 0.33	1.36 (0.90 to 2.06), 0.15	1.36 (0.90 to 2.06), 0.15
≥40	1.30 (0.79 to 2.13), 0.30	1.31 (0.74 to 2.31), 0.36	1.30 (0.73 to 2.28), 0.38
Gender male	**1.50 (1.04 to 2.16)**, 0.03	**1.78 (1.10 to 2.87)**, 0.017	**1.86 (1.19 to 2.93)**, 0.007
Injection drug use	**1.43 (1.00 to 2.02)**, 0.046	1.19 (0.69 to 2.06), 0.53	
HBs antigen positive	0.66 (0.38 to 1.15), 0.14		
Anti-HCV antibody positive	**1.46 (1.03 to 2.07)**, 0.03	**1.72 (1.01 to 2.91)**, 0.04	**1.89 (1.22 to 2.92)**, 0.004
AIDS before ART	0.71 (0.46 to 1.12), 0.14		
Baseline CD4 count (/mm^3)			
<100	1.00	1.00	1.00
100 to 199	**2.95 (1.97 to 4.41)**, <0.001	**2.95 (1.90 to 4.59)**, <0.001	**2.95 (1.90 to 4.60)**, <0.001
≥200	**3.53 (2.30 to 5.42)**, <0.001	**3.49 (2.15 to 5.65)**, <0.001	**3.48 (2.15 to 5.65)**, <0.001
NRTI			
AZT + 3TC or FTC	1.00	1.00	1.00
d4T + 3TC or FTC	**0.45 (0.29 to 0.67)**, <0.001	**0.51 (0.32 to 0.82)**, 0.005	**0.52 (0.33 to 0.83)**, 0.003
TDF + 3TC or FTC	0.86 (0.53 to 1.39), 0.53	1.30 (0.74 to 2.29), 0.36	1.30 (0.74 to 2.28), 0.37
Key drug			
NVP	1.00	1.00	1.00
EFV	**0.59 (0.42 to 0.84)**, 0.003	**0.53 (0.28 to 0.70)**, <0.001	**0.44 (0.28 to 0.70)**, <0.001

Impaired immune recovery was defined as failing to attain 100/μl increase in CD4 count in 24 months of the first-line ART. Factors provide $p > 0.1$ in univariate model were included in the multivariate models. Factors with *p*-value less than 0.1 in the univariate model were included in the multivariate models. Confidential intervals that do not overlap the null value of HR=1 are shown in bold. OR, odds ratio; CI, confidential interval; ART, antiretroviral therapy; HBs antigen, hepatitis B surface antigen; HCV, hepatitis C virus; NRTI, nucleos(t)ide reverse transcriptase inhibitors; AZT, zidovudine; d4T, stavudine; TDF, tenofovir; 3TC, lamivudine; FTC, emtricitabine; NNRTI, non-nucleoside reverse-transcriptase inhibitors; NVP, nevirapine; EFV, efavirenz.

This study had limitations. First, there was a lack of information about adherence to ART and other factors that could affect it such as alcohol consumption and mental health status [9]. Second, the baseline VL was not obtainable for all participants, and a high baseline VL is a known risk factor for VF. Third, the baseline CD4+ count was also unavailable for 365 patients (20%), which may obscure the precise effect of the baseline CD4+ count in our analyses. Fourth, the duration of ART at cohort enrolment was diverse, especially when the enrolment was started in 2007. Thus, the cohort may not include patients who died shortly after starting ART before 2007 and the survival outcome could be overestimated. Finally, both participating clinics were located in large urban referral hospitals in Hanoi, and it remains unclear whether our results are generalizable to other centres.

In conclusion, our study is the first study to describe the long-term probability of viral suppression and immune recovery during first-line ART in Vietnam. The viral suppression rate was 95.5% at 12 months and the survival rate without VF was maintained at 90% for 42 months. Younger age and HCV-antibody positivity were associated with early VF, while male sex, IDU, and HCV co-infection were associated with impaired immune recovery at 24 months. EFV-containing regimens provided\ better virologic outcomes and greater early CD4 recovery, compared to d4T-containing regimens, which had a higher rate of VF. Although we observed durable viral suppression, our results suggest that increased support is needed to help patients with IDU or HCV co-infection adhere to their ART. Our findings also support the current global trend toward wide-spread routine VL testing, which can facilitate more effective adherence support interventions.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Study concept and design: JT, SM, SH, KNV, SO; Collection and interpretation of data: JT, SM, DNT, HDNT, CDD, VVT, TTP, TNV. Drafting the manuscript: JT, SH; Statistical analysis: JT, SH; Obtained funding: SO.

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Social Support as a Key Protective Factor against Depression in HIV-Infected Patients: Report from large HIV clinics in Hanoi, Vietnam

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Depression is the most common mental health issue among people living with HIV/AIDS (PLWHA). This study explored how different types and sources of social support are associated with depression among HIV-infected patients in Vietnam. We carried out a cross-sectional survey on 1,503 HIV-infected patients receiving antiretroviral therapy at two HIV clinics in Hanoi in 2016. Depression was prevalent in 26.2% of participants. Higher score of social support, especially emotional/informational support and positive social interaction, showed significant association with lower depression rate. Although family was primary source of all types of social support, receiving emotional/informational support not only from family but also from outside of family correlated with a lower proportion of depression. In countries with constrained social resources and/or with family-oriented social structures, as in Vietnam, expanding social networks between HIV populations and society is a potentially important option for reducing depression.

The chronic nature of HIV has brought mental health issues to the fore as a critical problem in people living with HIV/AIDS (PLWHA). The most common mental health issue among PLWHA is depression, with the chance of developing a depressive disorder reportedly two to three times higher in PLWHA than in the general population[1,2]. Compared with PLWHA without depression, those with depression experience faster progression from HIV to AIDS[3,4], higher mortality[3,5], poorer adherence to antiretroviral therapy (ART)[6–8], and greater prevalence of HIV risk behaviors[9–11].

In Vietnam, a country with the fifth highest number of PLWHA in the Asia-Pacific region, the HIV epidemic is concentrated among high-risk groups (e.g., injection drug users [IDUs] and female sex workers)[12], and many such people live with multiple stigma of HIV and drug use and/or sex work[13,14]. Additionally, depression in Vietnam is often recognized as a sign of immaturity or weak personality[15]. Amid this climate, the shortage of mental health professionals limits PLWHA in accessing mental health services[15]. Moreover, Vietnam is now facing withdrawal of international donors' support for HIV-related services[16], and the cost for ART will be allocated to the national health insurance scheme. This political change may impose substantial financial burden on patients, in the form of premiums and out-of-pocket costs. For these reasons, the HIV population in Vietnam is facing, or will be facing, greater risk of depression. However, few studies have addressed this issue in Vietnam[6,17–20]. These studies have had several limitations, such as relatively small sample size[18,19], focusing on only a subgroup of the HIV population (e.g., males or sex workers)[18,20], and using poorly validated instruments[17].

There is a large body of evidence that social support plays a beneficial role in health[21–24]. Social support by definition is assistance people receive, or perceive, from their social networks. It is a multidimensional concept comprising *types* and *sources*[25]. Major types of social support include emotional support (e.g., empathy, trust, or care), informational support (e.g., advice, suggestions, or information), tangible support (e.g., practical help, assistance, or financial support), and positive social interaction or social companionship (e.g., spending time with others in

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leisure and recreational activities)[26–28]. Sources of social support include family members, friends, neighbors, and colleagues. These two dimensions of social support may affect health and well-being in various ways[29–32].

Compared with wealthy countries, low- and middle-income countries such as Vietnam have very limited social resources, such as support groups, available for HIV patients. Additionally, notably in Vietnam and often in other Asian countries, family is viewed as an extension of the self—a notion based on Confucian tradition—and family ties are much more interdependent and tightly knit than those in many Western countries[33,34]. Such countries may present no other options for HIV patients to seek support or assistance than through family when they are facing trouble. The perceptions and effectiveness of social support are closely interwoven into the socio-cultural context. Although a number of studies have shown a protective role of social support in depression in the HIV population[35–39], we still do not have enough evidence on what social support available, and from whom, are most effective at protecting HIV patients against depression in countries with constrained social resources and/or with family-oriented cultures. Therefore, this study aimed to examine how different types and sources of social support are associated with depression among PLWHA in one such country: Vietnam.

Methods

Study design and study subjects. We conducted a self-administered questionnaire survey using a hospital-based cohort of PLWHA (aged ≥ 18 years). All participants were HIV-infected patients receiving ART. This "Hanoi cohort" was established in 2007 at two HIV outpatient clinics: National Hospital of Tropical Diseases (NHTD) and Bach Mai Hospital (BMH) in Hanoi. These are the largest referral clinics in Hanoi and are located next to each other.

A pilot survey was conducted for 2 days in December 2015, recruiting 67 patients who visited NHTD or BMH to verify the survey contents and test research procedures. We then conducted the actual survey during patients' monthly visits between January and December 2016. We assigned a clinical staff member (nurse or social worker) to provide appropriate support for the participants. We excluded patients who participated in the pilot survey and who did not complete the questionnaire on depression from the present analyses.

Measurements. *Depression.* Depression was evaluated using CES-D[33]. CES-D is a widely used self-reporting scale for measuring depressive symptoms that participants experience[34,35]. There is strong evidence for both its reliability and validation in Vietnam's HIV population, with Cronbach's alpha of 0.81, and sensitivity and specificity of 79.8% and 83.0%, respectively, at the cut-off score of 16[19].

CES-D consists of 20 items. Responses were given on a four-point scale ranging from 0 (rarely or none of the time) to 3 (most or almost all the time), except for four items that were positively worded and scored in reverse. We used the Vietnamese version of CES-D, which was already available, and in this study defined a CES-D score of ≥16 as indicating depression. This cut-off score has been proven optimal for assessing depression in Vietnam's HIV population[19].

Social support. Types of social support were evaluated using the Medical Outcome Study Social Support Survey (MOS-SSS)[36]. This is a self-administered scale developed to measure perceived availability of types of social support, regardless of their sources. MOS-SSS consists of 19 items representing four types of social support; emotional/informational support (eight items), tangible support (four items), affectionate support (three items), positive social interaction (three items), and additional item (one item). The score for each item ranges from 0 (rarely or none of the time) to 5 (all of the time). MOS-SSS has been translated into various languages and adapted to different cultures and contexts[37–42], however, because a Vietnamese version was not available, the English version was translated and then back-translated for use in this study to verify the accuracy of the translation. Additionally, in the pilot survey, respondents were asked to indicate any wording or expressions they did not understand or found unacceptable or offensive in view of cultural norms. After the pilot survey, an expert bilingual panel, including the original translator and health professionals, decided on the final version. Then, using the data from the main survey, Cronbach's alpha was calculated to evaluate internal consistency, and confirmatory factor analysis was performed to examine the construct validity of Vietnamese version. Summed scores in each type of social support were used as continuous variables and categorical variables (quartiles) in the descriptive analyses, and as continuous variables in other analyses. Additional items were excluded from the analyses.

Information on sources of each type of social support in MOS-SSS was added to the questionnaire. Considering the prevalent family-oriented culture in Vietnam, we categorized sources of social support focusing on family and divided into the following groups: family only, family and others (i.e., partner, friends, medical staff, and others), lack of family (i.e., receiving support only from others), and none.

Demographics and HIV-related factors. The following data on demographic and HIV-related factors were collected: sex, age, IDU history, number of HIV-related symptoms, duration from HIV diagnosis, duration from ART initiation, history of EFV usage, latest CD4 count (/μl), and latest plasma viral load (pVL) (copies/ml). Age was divided into two categories: <35 years and ≥35 years. History of IDU was divided into three categories: current IDU (used injection drugs in the past 6 months), former IDU (history of injection drug use, but not used in the past 6 months), and non-IDU (never used injection drugs). HIV-related symptoms were measured using the 20-item HIV Symptom Index[43]. The total number of symptoms was used as a continuous variable. Duration from HIV diagnosis and from ART initiation were divided into the following categories: <1 year, 1–2 years, 3–4 years, and ≥5 years. History of EFV usage was divided into three categories: non-user, former user, and current user. CD4 count and pVL were tested in a laboratory, and data from the last clinic visit before the survey were obtained. These were divided into groups: <350 and ≥350, and <20 and ≥20.

Social factors. Information on social factors, other than social support, that was obtained included: residence, marital status, number of household members, educational attainment, employment, individual income, health insurance, and disclosure status. Residence was given as either Hanoi or others. Marital status was divided into four categories: not married, no partner; not married, but have a partner; married; and others (e.g., divorced or widowed). Number of household members was used as a continuous variable. Educational attainment was divided into three groups: low (never went to school, primary school, or junior high school), middle (high school), and high (vocational school/college or university). Employment was categorized as not employed, employed, or retired. Individual income was divided into the following categories: low (<1,500,000 Vietnamese dong [VND]), middle (1,500,000–4,999,999 VND), and high (≥5,000,000 VND). Health insurance and HIV disclosure status were evaluated dichotomously.

Statistical analysis and ethics statement. We analyzed the prevalence of depression (CES-D ≥ 16) in accordance with the types and sources of social support the respondents perceived. Logistic regression analyses were then used to examine the statistical associations between social support and depression.

In the logistic regression analyses, we first focused on *types* of social support in relation to depression. In univariate models, we calculated the odds ratio (OR) with the 95% confidence interval (95%CI) for types of social support and other explanatory variables. A multivariate model was then developed to calculate the adjusted OR and 95%CI. Given the exploratory nature of the present analyses, we used a stepwise selection method for all variables (inclusion and exclusion criteria = 0.2 for each) in the multivariate models to improve statistical power. We employed four types of social support in the multivariate model to investigate which among them were predominantly associated with depression. In sensitivity analysis, considering the strong correlation between these four types, we developed four multivariate models in which each type was used individually to confirm the results of the prior model. Next, the *sources* of each type of social support as related to depression were further investigated. In this analysis, we used the sources of each social support type individually in the model.

By way of supplementary analyses, we calculated the adjusted OR and 95%CI of four variables—sex, age, IDU, and history of EFV usage—in every multivariate model by performing the stepwise selection method using forced entry of these variables. The variables were of interest because they had shown an association with depression in previous reports[15,20,44–48].

All analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). All tests were two-sided, with the significance level set at 5%. Missing data were excluded from the analyses.

Ethics statement. The study was approved by the Human Research Ethics Committee of the National Center for Global Health and Medicine (reference: NCGM-G-001845-00), BMH (reference: 19/BM-HDDD), and NHTD (reference: 12/HDDD-NDTU). We performed this study in accordance with the Japan Ethical Guidelines for Medical and Health Research Involving Human Subjects issued by Japan Ministry of Health, Labor and Welfare, in Dec 2014. Each participant provided written informed consent. Data were anonymized.

Results

Study participants. Since October 2007, 2,198 patients had registered for the Hanoi cohort, and 1,773 were still enrolled and underwent follow up in December 2015. Of those, 270 were excluded from the analyses: 134 who did not participate in the survey owing to their having no opportunity or time to take it, 67 from the pilot survey, and 69 who did not complete the CES-D. Thus, the present analysis included 1,503 patients.

Table 1 shows the respondents' characteristics. The median age (interquartile range [IQR]) was 38 (33–42) years. In all, 22.2% of the respondents had a history of IDU. Among them, 1.5% were current users. Moreover, more than half of the respondents had been receiving ART for ≥5 years and 72.4% had been exposed to efavirenz (EFV).

Social support and depression. Table 2 shows the prevalence of depression (CES-D ≥ 16) in accordance with the types and sources of social support the participants perceived. The overall prevalence of depression in this population was 26.2% (23.0% in men, 30.8% in women). A linear inverse association between the score of each social support type and depression was observed. Furthermore, more than 80% of patients were receiving each type of social support from their family. Only 30–40% of patients were receiving social support from someone outside their family, except for emotional/informational support (58.4%).

Types of social support and other factors. Types of social support were evaluated using the aforementioned Vietnamese version of MOS-SSS. In the main survey, this scale showed good internal consistency with Cronbach's alpha, 0.95, for the overall score, and 0.90–0.93 for the four sub-scales. In confirmatory factor analysis, an original four-factor model of MOS-SSS provided the best-fitting structure, with a comparative fit index of 0.96 and adjusted goodness of fit index of 0.89. Construct validity of the Vietnamese version was thereby confirmed (Supplementary Figure S1).

Using this scale, we first investigated statistical association between four types of social support and depression. Table 3 shows the results from univariate and multivariate logistic regression models. In the multivariate model, sex, number of HIV-related symptoms, duration from HIV diagnosis, number of household members, employment, individual income, emotional/informational support, and positive social interaction were selected via a stepwise selection method and included in the final model. Among these variables, higher number of HIV-related symptoms (OR = 1.35, 95%CI: 1.25–1.46 per increase of one in number of symptoms), duration from HIV diagnosis (<1 year) (OR = 7.98, 95%CI: 1.48–43.11 vs. ≥ 5 years), and being unemployed (OR = 1.76, 95%CI: 1.18–2.61 vs. employed) were associated with higher proportion of having depression. However, high individual income (OR = 0.61, 95%CI: 0.38–0.98 vs. low individual income), and higher score for

variables	n	%
Demographics		
Gender		
Male	903	60.08
Female	600	39.92
Age, median (IQR) years	38 (33, 42)	
<35 years	460	30.61
≥35 years	1043	69.39
HIV-related factors		
History of IDU[a]		
Current IDU	23	1.53
Former IDU	311	20.69
Non IDU	1167	77.64
N/A	2	0.13
Number of HIV-related symptoms, median (IQR)	1 (0, 2)	
Duration from HIV diagnosis		
<1 year	11	0.73
1–2 year	134	8.92
3–4 year	331	22.02
≥5 years	1027	68.33
Duration from ART initiation		
<1 year	65	4.32
1–3 year	174	11.58
3–4 year	373	24.82
≥5 years	891	59.28
History of EFV usage		
Non-user	415	27.61
Former user	95	6.32
Current user	993	66.07
Latest CD4 count, median (IQR) (/μl)[b]	442 (322, 574)	
<350	437	29.08
≥350	1065	70.86
N/A	1	0.07
Latest plasma viral load (copies/ml)[b]		
<20	1261	83.9
≥20	240	15.97
N/A	2	0.13
Social factors		
Residence		
Hanoi	633	42.12
Others	866	57.62
N/A	4	0.27
Marital Status		
Not married and not having a partner	111	7.39
Not married but having a partner	48	3.19
Married	1296	86.23
Others (divorced, widow, etc.)	48	3.19
Number of household members, median (IQR)	4 (3, 4)	
Educational attainment[c]		
Low	421	28.01
Middle	467	31.07
High	615	40.92
Employment		
Not employed	332	22.09
Employed	1093	72.72
Retired	69	4.59
N/A	9	0.60
Individual income[d]		

Continued

variables	n	%
Low	382	25.42
Middle	719	47.84
High	319	21.22
N/A	83	5.52
Health insurance		
No	280	18.63
Yes	1212	80.64
Unknown	11	0.73
Disclosure status		
No	69	4.59
Yes	1433	95.34
N/A	1	0.07

Table 1. Characteristics of participants. N/A: missing value. [a]IDU: injection drug user; current IDU: used injection drugs in the past 6 months; former IDU: history of injection drug use, but not used in the past 6 months; non-IDU: never used injection drugs. [b]Data at last clinic visit before the survey. [c]Low: never went to school, primary school, or junior high school; Middle: high school; High: vocational school/college or university. [d]Low: <1,500,000 Vietnamese dong (VND); Middle: 1,500,000–4,999,999 VND; High: ≥5,000,000 VND.

emotional/informational support and positive social interaction (OR = 0.94, 95%CI: 0.92–0.96, OR = 0.93, 95%CI: 0.89–0.98 per one-point increase in MOS-SSS score, respectively) were protectively associated with depression.

In the sensitivity analysis, wherein each type of social support was used individually in the multivariate model, the variables selected via the stepwise method were the same as those in the primary model, except that sex was not selected. In these models, all types of social support were significantly associated with lower proportion of having depression (OR = 0.92, 95%CI: 0.91–0.94 for emotional/informational support, OR = 0.91, 95%CI: 0.88–0.93 for tangible support, OR = 0.88, 95%CI: 0.85–0.91 for affectionate support, OR = 0.85, 95%CI: 0.82–0.88 for positive social interaction, per one-point increase in MOS-SSS score, respectively) (Table 4). The direction of effect and statistical significance of other explanatory variables in these models did not change from the primary model, except that the number of household members and duration from HIV diagnosis (3–4 years) showed statistical significance in all models.

Sources of social support. Next, we investigated the sources of each social support type for its relation with depression. Table 5 shows the results of univariate and multivariate logistic regression models. We used the sources of each social support type individually in four multivariate models. In these models, the variables selected via the stepwise method were the same as those in the primary model. With regard to tangible support, affectionate support, and positive social interaction, those not receiving such support from anyone (OR = 2.04, 95%CI: 1.07–3.87; OR = 2.29, 95%CI: 1.44–3.65; and OR = 2.15, 95%CI: 1.19–3.88, respectively) nor from their family (OR = 2.78, 95%CI: 1.49–5.18; OR = 3.05, 95%CI: 1.87–4.97; and OR = 1.76, 95%CI: 1.19–2.62, respectively), showed higher probability of having depression compared with those receiving such support from their family. However, the source of emotional/informational support showed a unique association with depression. Although not receiving emotional/informational support from one's family was not significantly associated with depression, those receiving such support from both their family and others showed a lower rate of depression compared with those receiving it only from their family (OR = 0.58, 95%CI: 0.44–0.77).

Supplementary analysis. We calculated the adjusted OR and 95%CI for four variables—sex, age, IDU, EFV usage—in every multivariate model by applying the forced entry of these variables in the stepwise selection method. Although being female and being a current or former IDU were associated with higher rate of having depression in all the models, and being a current EFV user always showed a protective association with depression, these were not statistically significant. The direction of effect of age and being a former EFV user changed from model to model, and the association did not show statistical significance in any of the models.

Discussion

The prevalence of depression was 26.2% in the present study sample. After controlling by various socio-demographic factors, social support was found to have a significant association with lower depression rate. Among four types of social support, emotional/informational support and positive social interaction were found predominantly associated with a lower proportion of people with depression. Although family was the primary source of all types of social support, those receiving emotional/informational support not only from family but also from people outside that family showed a lower proportion of having depression.

The prevalence of depression found in this study (26.2%) was relatively lower than that in previous studies targeting Vietnam's HIV population, and using the same cut-off score for CES-D (36.5–78.0%)[6,18,19]. However, the prevalence in the present study was near that reported for Vietnam's general population (24.3%)[49]. This could be because we included patients in a stabilized HIV condition (e.g., 71% with CD4 ≥ 350) and who were receiving

Variables	n	%	CES-D ≥ 16 n (%)
All participants	1503	100.0	393 (26.15)
Types of social support[a]			
Emotional/Informational support (8–40), median (IQR)	30 (23, 35)		
Low (8–22)	314	20.89	146 (46.50)
Medium-low (23–29)	393	26.15	126 (32.06)
Medium-high (30–34)	364	24.22	83 (22.80)
High (35–40)	399	26.55	27 (6.77)
N/A	33	2.20	11 (33.33)
Tangible support (4–20), median (IQR)	17 (14, 20)		
Low (4–13)	354	23.55	162 (45.76)
Medium-low (14–16)	303	20.16	88 (29.04)
Medium-high (17–19)	296	19.69	60 (20.27)
High (20)	537	35.73	78 (14.53)
N/A	13	0.86	5 (38.46)
Affectionate support (3-15), median (IQR)	12 (9, 15)		
Low (3–8)	327	21.76	147 (44.95)
Medium-low (9–11)	270	17.96	84 (31.11)
Medium-high (12–14)	434	28.88	100 (23.04)
High (15)	456	30.34	56 (12.28)
N/A	16	1.06	6 (37.50)
Positive social interaction (3-15), median (IQR)	12 (9, 15)		
Low (3-8)	299	19.89	149 (49.83)
Medium-low (9–11)	374	24.88	121 (32.35)
Medium-high (12–14)	414	27.54	69 (16.67)
High (15)	410	27.28	52 (12.68)
N/A	6	0.40	2 (33.33)
Sources of each social support type			
Source of emotional/informational support			
Only family	603	40.12	187 (31.01)
Family and others[b]	735	48.90	145 (19.73)
Lack of family (only from others[b])	143	9.51	53 (37.06)
None	19	1.26	6 (31.58)
N/A	3	0.20	2 (66.67)
Source of tangible support			
Only family	876	58.28	226 (25.8)
Family and others[b]	508	33.80	115 (22.64)
Lack of family (only from others[b])	58	3.86	26 (44.83)
None	55	3.66	23 (41.82)
N/A	6	0.40	3 (50.00)
Source of affectionate support			
Only family	837	55.69	196 (23.42)
Family and others[b]	460	30.61	107 (23.26)
Lack of family (only from others[b])	95	6.32	41 (43.16)
None	111	7.39	49 (44.14)
Source of positive social interaction			
Only family	724	48.17	174 (24.03)
Family and others[b]	525	34.93	118 (22.48)
Lack of family (only from others[b])	179	11.91	66 (36.87)
None	73	4.86	35 (47.95)
N/A	2	0.13	0 (0.00)

Table 2. Prevalence of depression by type and source of social support. N/A: missing value. [a]categories were developed using quantiles of the score in each type of social support. [b]others: partner, friends, medical staff, and others.

variables	Univariate model		Multivariate model[a] (n = 1,329)	
	OR (95%CI)	*p*	OR (95%CI)	*p*
Sex				
Male	1.00	<0.001	1.00	0.15
Female	1.49 (1.18–1.88)		1.24 (0.93–1.65)	
Age (years)				
<35	1.13 (0.88–1.45)	0.33	—	
≥35	1.00		—	
History of IDU[b]				
Current IDU	2.60 (1.14–5.96)	0.07	—	
Former IDU	0.95 (0.71–1.27)		—	
Non IDU	1.00		—	
Number of HIV-related symptoms	1.41 (1.33–1.50)	<0.001	1.35 (1.25–1.46)	<0.001
Duration from HIV diagnosis (years)				
<1	2.55 (0.77–8.43)	0.13	7.98 (1.48–43.11)	0.03
1–2	1.12 (0.75–1.69)		1.35 (0.82–2.22)	
3–4	1.31 (0.99–1.72)		1.33 (0.95–1.87)	
≥5	1.00		1.00	
Duration from ART initiation (years)				
<1	1.00	0.37	—	
1–2	1.02 (0.53–1.94)		—	
3–4	1.18 (0.65–2.14)		—	
≥5	0.93 (0.52–1.64)		—	
History of EFV usage				
Non-user	1.00	0.09	—	
Former user	1.21 (0.75–1.95)		—	
Current user	0.81 (0.62–1.04)		—	
Latest CD4 count (/μl)[c]				
<350	1.00	0.34	—	
≥350	1.13 (0.88–1.46)		—	
Latest plasma viral load (copies/ml)[c]				
<20	1.00	0.25	—	
≥20	1.20 (0.88–1.62)		—	
Residence				
Hanoi	1.00	0.10	—	
Others	1.22 (0.96–1.54)		—	
Marital Status				
Not married, no partner	1.84 (1.23–2.76)	0.02	—	
Not married, but have a partner	1.38 (0.74–2.56)		—	
Married	1.00		—	
Others (divorced, widowed, etc.)	1.25 (0.66–2.35)		—	
Number of household members	0.76 (0.68–0.90)	<0.001	0.84 (0.70–1.00)	0.05
Educational attainment[d]				
Low	1.66 (1.25–2.20)	<0.01	—	
Middle	1.33 (1.00–1.76)		—	
High	1.00		—	
Employment				
Not employed	2.41 (1.86–3.13)	<0.001	1.76 (1.18–2.61)	0.02
Employed	1.00		1.00	
Retired	0.98 (0.54–1.76)		0.98 (0.47–2.05)	
Individual income[e]				
Low	1.00	<0.001	1.00	0.11
Middle	0.56 (0.43–0.73)		0.86 (0.59–1.24)	
High	0.31 (0.22–0.45)		0.61 (0.38–0.98)	
Health insurance				
No	1.14 (0.86–1.53)	0.37	—	

Continued

variables	Univariate model		Multivariate model[a] (n = 1,329)	
	OR (95%CI)	*p*	OR (95%CI)	*p*
Yes	1.00		—	
Disclosure status				
No	1.08 (0.63–1.86)	0.78	—	
Yes	1.00		—	
Emotional/Informational support	0.91 (0.90–0.93)	<0.001	0.94 (0.92–0.96)	<0.001
Tangible support	0.89 (0.87–0.91)	<0.001	—	
Affectionate support	0.85 (0.83–0.88)	<0.001	—	
Positive social interaction	0.81 (0.79–0.84)	<0.001	0.93 (0.89–0.98)	0.01

Table 3. Odds ratios and 95% confidence intervals of types of social support and other variables in univariate and multivariate logistic regression models. OR: odds ratio; 95%CI: 95% confidence interval; *p*: p-value. -: included in multivariate model, but not selected via stepwise selection methods for the final model. [a]Stepwise selection method for all variables was used (inclusion and exclusion criteria = 0.2 for each). [b]IDU: injection drug user, current IDU: used injection drugs in the past 6 months); former IDU: history of injection drug use, but not used in the past 6 months; non-IDU: never used injection drugs. [c]Data at last clinic visit before the survey. [d]Low: never went to school, primary school, or junior high school; Middle: high school; High: vocational school/college or university. [e]low: <1,500,000 Vietnamese dong (VND); middle: 1,500,000–4,999,999 VND; high: ≥5,000,000 VND.

HIV treatment in tertiary-level facilities. Our result may be a hopeful sign suggesting engagement in continuous HIV treatment and care with better quantity and quality of resources could help patients maintain a healthy mental status at rates comparable to those of the general population.

Social support was found significantly associated with a lower depression rate. This association was stronger than that with previously reported risk factors of depression (e.g., sex, age, IDU, EFV usage), and did not change even after controlling by various socio-demographic factors. Although all four types of social support examined individually showed a protective association with depression, this association was most prominent between emotional/informational support and positive social interaction, and depression. This is somewhat consistent with previous findings, which suggested social support, especially emotional support, buffers the deleterious influences of stressful events and mitigates the risk of depression[50–53]. The chronic course of HIV infection requires patients to cope with various forms of psychosocial stress associated with opportunistic infection, side effects of ART, and social prejudice and discrimination[54]. Therefore, our findings may suggest that, even though most participants had been receiving ART for a long period and their health status was stable, those without social support tended to be facing substantial psychological stress and were at a greater risk of contracting depression. For such a population, compared with practical support, emotional/informational support and positive social interaction may be more effective support forms for mitigating their stress, and thereby safeguarding against depression. Additionally, the importance of positive social interaction found in this study may reflect a limited social network between HIV populations and general society. In Vietnam, drug users and sex workers have been critically labeled as "social evils" in relation to HIV transmission[15]. Social discrimination and prejudice against the HIV population resulted in discouraging such people from disclosing their HIV status to anyone outside their family[15,55]. Indeed, in our study, while more than 90% of patients had disclosed their status to their family, only 14% had done so to their friends.

As previously reported, we also found family to be the vital source of all types of social support among HIV patients; we found participants who lacked tangible support, affectionate support, and positive social interaction from their family had a higher probability of having depression. Interestingly, however, regarding emotional/informational support, lack of family support itself was not associated with depression. Rather, receiving such support not only from family, but also from people outside the family, was protectively associated with depression. This result may have important implications for social support intervention for mental health. Although it has been well-known that forms of family-based intervention, such as involvement of family in HIV therapy or provision of educational programs for family, could help foster patients' mental health[56–58], people outside the family may be also important contributors, even in family-oriented societies. With such social structures as in Vietnam, those without family support may face difficulty and stress in disclosing their HIV status to their family and seeking support from them. Considering that close relationships represented by family ties may be a potential source of stress, and may have a deleterious influence on health[59–61], programs aimed at bolstering social support by expanding social networks to people outside the family, rather than by strengthening family support, may be more effective at fostering mental health in such cases.

Notably, our study contained a unique system for HIV treatment and care, in which treatment groups were organized. Professionals formed groups of 10–30 previously unacquainted HIV-infected patients who started ART in the same month. Subsequently, medical follow-up schedules, adherence counseling sessions, and other activities were arranged. This system may naturally create social ties between patients, and foster peer support among them. It may also have contributed to the lower prevalence of depression found in this study, and be a potential and already existing resource to strengthen social support.

Our study showed low prevalence of depression among HIV patients engaged in continuous HIV treatment and care with appropriate resources. However, considering the association of unemployment and low individual income with depression found in this study, the shifting financial burden for HIV services may exert further

Type of social support	OR (95%CI)	*p*
Emotional/Informational support	0.92 (0.91–0.94)	<0.001
Tangible support	0.91 (0.88–0.93)	<0.001
Affectionate support	0.88 (0.85–0.91)	<0.001
Positive social interaction	0.85 (0.82–0.88)	<0.001

Table 4. Sensitivity analyses: odds ratios and 95% confidence intervals of each type of social support individually used in four multivariate logistic regression models. OR: odds ratio; 95%CI: 95% confidence interval; *p*: p-value. Odds ratio was calculated in four multivariate models in which each type was used individually. Odds ratios were adjusted by number of HIV-related symptoms, duration from HIV diagnosis, number of household members, employment, and individual income. Except for types of social support, higher number of HIV-related symptoms, duration from HIV diagnosis (<1 year and 3–4 years vs. ≥5 years), and unemployment were associated with higher probability of depression; higher number of household members and high individual income were protectively associated with depression in all models.

	Univariate model		Multivariate model	
Source of support	OR (95%CI)	*p*	OR (95%CI)	*p*
Source of emotional/informational support				
Only family	1.00	<0.001	1.00	<0.001
Family and others	0.55 (0.43–0.70)		0.58 (0.44–0.77)	
Lack of family (only from others)	1.31 (0.90–1.92)		1.08 (0.68–1.69)	
None	1.03 (0.38–2.74)		0.77 (0.23–2.55)	
Source of tangible support				
Only family	1.00	<0.001	1.00	<0.01
Family and others	0.84 (0.65–1.09)		0.93 (0.70–1.24)	
Lack of family (only from others)	2.34 (1.36–4.01)		2.78 (1.49–5.18)	
None	2.07 (1.19–3.61)		2.04 (1.07–3.87)	
Source of affectionate support				
Only family	1.00	<0.001	1.00	<0.001
Family and others	0.99 (0.76–1.30)		0.97 (0.71–1.32)	
Lack of family (only from others)	2.48 (1.61–3.84)		3.05 (1.87–4.97)	
None	2.59 (1.72–3.88)		2.29 (1.44–3.65)	
Source of positive social interaction				
Only family	1.00	<0.001	1.00	<0.001
Family and others	0.92 (0.70–1.20)		0.87 (0.64–1.18)	
Lack of family (only from others)	1.85 (1.30–2.62)		1.76 (1.19–2.62)	
None	2.91 (1.78–4.75)		2.15 (1.19–3.88)	

Table 5. Odds ratio and 95% confidence intervals of sources of each social support type individually used in univariate and multivariate logistic regression models. OR: odds ratio; 95%CI: 95% confidence interval; *p*: p-value. Others: partner, friends, medical staff, and others. Odds ratios was calculated when using each source of social support one by one in the multivariate models. Odds ratios were adjusted by sex, number of HIV-related symptoms, and duration from HIV diagnosis, number of household members, employment, and individual income all model. Except for sources of social support, higher number of HIV-related symptoms, duration from HIV diagnosis (<1 year and 3–4 years vs. ≥5 years), and unemployment were associated with depression; higher number of household members and high individual income were protectively associated with depression in all models.

psychological burden on patients, and increase their risk of depression. Against this backdrop, this is the first investigation on two dimensions of social support (types and sources) and depression in Vietnam, using a large sample of the HIV-infected population. We found potential effectiveness of social support as an alleviator of depression, and recognized the importance of people outside the family as a source of such support. The findings of our study could provide useful information for future mental health strategies in other resource-constrained settings and in family-oriented societies. However, there are several limitations in this study. First, we adopted a cross-sectional design, which limits causal inferences for the associations found. The association between lack of social support and depression found herein may reflect reverse causation (i.e., people with depression do not seek social support). Second, our study sites were two large referral HIV clinics located in a main city; therefore, the characteristics of the participants may not accurately represent Vietnam's entire HIV population. Third, CES-D is not a diagnostic instrument. Our findings can only be interpreted in association with depressive symptomatology. Fourth, with the forward- and back-translation processes and the results of Cronbach's alpha and confirmatory

factor analysis, the content and the construct validity of the Vietnamese version of MOS-SSS were reinforced to some extent, but the scale's cultural adaptation and validity should be further explored using more standardized methodologies including the guidance developed by the Translation and Cultural Adaptation working group in the International Society for Pharmacoeconomics and Outcomes Research[62]. Finally, we did not assess supporters' relationships and dynamics in relation to depression. Such information is needed for formulating a strategic plan to address depression.

In conclusion, the prevalence of depression found in this study was low compared with that in previous reports. Even in countries with constrained social resources and/or with family-oriented culture, people outside the family are potentially important in promoting mental health in HIV-infected patients. It is important to expand social networks between HIV populations and general society, especially for those with less family support.

Data availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

Conceived and designed the study: S.M., J.T., D.M., S.O. Corrected and analyzed the data: S.M., K.Y., K.T., C.D.D., D.T.N., H.D.T.N., K.V.N. Wrote the paper: S.M.

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PROKARYOTES

Complete Genome Sequence of a *Mycobacterium tuberculosis* Strain Belonging to the East African-Indian Family in the Indo-Oceanic Lineage, Isolated in Hanoi, Vietnam

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ABSTRACT The East African-Indian (EAI) family of *Mycobacterium tuberculosis* is an endemic group mainly observed in Southeast Asia. Here, we report the complete genome sequence of an *M. tuberculosis* strain isolated as a member of the EAI family in Hanoi, Vietnam, a country with a high incidence of tuberculosis.

The incidence of tuberculosis in Vietnam was estimated to be 128 per 100,000 individuals in 2015 (1). In Hanoi, the capital city of the country, a population-based study to determine the genetic background of *Mycobacterium tuberculosis* was conducted by our group from 2007 to 2009, and 465 clinical strains were isolated (2). Although Beijing family strains belonging to the East Asian lineage (lineage 2) (3, 4) were predominant (58.5%) in Hanoi, the East African-Indian (EAI) family belonging to an ancestral Indo-Oceanic lineage (lineage 1), isolated from Southeast Asian countries and other regions (5), has also been isolated (17.6%) in the same area (2). In Vietnam, the influence of EAI family strains may exceed that of Beijing family strains in rural areas, indicating that the EAI family may have been indigenous to this region before the Beijing family started to spread (6, 7).

Here, we describe the complete genome sequence of HN-024, an EAI4-VNM strain of *M. tuberculosis*, defined by *in silico* spoligotyping. This strain was isolated from a 58-year-old Vietnamese woman before initial treatment for tuberculosis in 2007 (2). The strain showed no resistance to rifampin, isoniazid, streptomycin, ethambutol, or pyrazinamide by drug susceptibility testing and a pyrazinamidase assay. A PacBio RS II instrument (Pacific Biosciences, CA) was used to determine the complete genome sequence. Long-read sequences were obtained (399.8 Mb, with 4,821 bp as the average length of read inserts) and assembled with Hierarchical Genome Assembly Process (HGAP) version 3. Consequently, a single contig over 4.4 Mb in length was assembled. The contig was polished by a mapping analysis of 300-bp paired-end reads obtained by MiSeq sequencing (Illumina, CA), using CLC Genomics Workbench (Qiagen, CA, USA). After polishing, two regions, including repetitive structures by remapping analysis, remained, and their nucleotide sequences were directly determined by Sanger sequencing for confirmation.

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Finally, the complete genome sequence was 4,399,916 bp, with 65.6% G+C content. These values were similar to previously reported complete genomes, such as those of H37Rv (a reference strain, with 4,411,532 bp and 65.6% G+C content) (8) and EAI5 (a member of the EAI family, with 4,391,174 bp and 65.6% G+C content) (9). Prior to submission to DDBJ, D-FAST (10), a pipeline, including annotation by PROKKA version 1.11 (11), was applied to the genome sequence, which predicted 4,027 protein-coding regions and 52 tRNAs. To improve the compatibility and usability of the new sequence, the original annotation by D-FAST was modified to include locus tags and gene names of the H37Rv genome (GenBank accession no. AL123456.3), according to reciprocal BLASTP best hits by standalone BLAST+ (version 2.2.29) (12).

Because the genome sequence reported in this study was obtained from a typical Vietnamese EAI strain, it could serve as a reference for comparative genomics of *M. tuberculosis* and may provide clues to elucidate the history of the spread of EAI family strains in Vietnam.

Accession number(s). This whole-genome sequencing study has been deposited at DDBJ/ENA/GenBank under the accession number AP018033. The version described in this paper is the first version.

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Complete Genome Sequences of Three Representative *Mycobacterium tuberculosis* Beijing Family Strains Belonging to Distinct Genotype Clusters in Hanoi, Vietnam, during 2007 to 2009

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ABSTRACT We present here three complete genome sequences of *Mycobacterium tuberculosis* Beijing family strains isolated in Hanoi, Vietnam. These three strains were selected from major genotypic clusters (15-MIRU-VNTR) identified in a previous population-based study. We emphasize their importance and potential as reference strains in this Asian region.

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Tuberculosis remains a major health threat worldwide, with 6.1 million new cases in 2015 and 49 million deaths between 2000 and 2015 (1). Of the seven major lineages of *Mycobacterium tuberculosis* in the world, Beijing family strains belong to lineage 2, the East-Asian lineage, and are endemic to the eastern part of Asia (2). This family has attracted the attention of many researchers because of its associated drug resistance, relapse, and transmissibility.

Vietnam has a high incidence of tuberculosis (1), with a high prevalence of Beijing family strains (3–5). In a previous population-based study in Hanoi, the capital of Vietnam, between 2007 and 2009 (4), we analyzed genotypic clusters defined by identical patterns of variable numbers of tandem repeat polymorphisms (15-MIRU-VNTR) to assess recent transmission (data not shown).

Here, we report the complete genome sequences of representative *M. tuberculosis* Beijing family strains (HN-205, HN-321, and HN-506) in the three major genotypic clusters. All strains were isolated from Vietnamese patients living in Hanoi (4). Phylogenetically, HN-205 and HN-506 belonged to the modern Beijing subfamily, ST10 (6, 7), whereas HN-321 belonged to the ancient Beijing subfamily, ST19/25.

Long-read sequences obtained using a PacBio RS II instrument (Pacific Biosciences, USA) were assembled with the Hierarchical Genome Assembly Process (HGAP) version 3. Consequently, a single contig was assembled in each of the strains. These contigs were polished by mapping analysis of 300-bp paired-end reads by MiSeq sequencing (Illumina, USA), using CLC Genomics Workbench version 7.5.2 (Qiagen, USA). After polishing, sequence regions with low coverage (<5) by remapping analysis were confirmed by Sanger sequencing.

The lengths of the complete genome sequences were 4,411,033 bp for HN-205, 4,421,540 bp for HN-321, and 4,413,362 bp for HN-506. G+C contents were all 65.6%.

Prior to submission to DDBJ, D-FAST (8), a pipeline that includes annotation by Prokka version 1.11 (9), was applied, which predicted 4,059 genes for HN-205, 4,066 genes for HN-321, and 4,064 genes for HN-506, in addition to 52 tRNAs for all strains. The original annotation by D-FAST was modified to include locus tags and gene names of the H37Rv genome sequence (DDBJ no. AL123456.3), according to reciprocal BLASTp best hits by stand-alone BLAST+ version 2.2.29 (10).

Despite a lack of previous tuberculosis treatment history, conventional drug susceptibility tests for the first-line drugs reveled that HN-321 and HN-506 were both resistant to isoniazid (data not shown), with a *kat*G gene mutation at codon 315 (Ser to Thr). Streptomycin resistance was identified only in HN-321, with an *rps*L mutation at codon 43 (Lys to Arg). HN-205 was susceptible to all antibiotics.

As reference strains, the complete genome sequences reported here will be helpful in public health and in studies on transmission and drug resistance of Beijing strains in Vietnam.

Accession number(s). This whole-genome sequencing study has been deposited at DDBJ/ENA/GenBank under the accession numbers AP018034 (HN-205), AP018035 (HN-321), and AP018036 (HN-506). The versions described in this paper are the first versions.

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GBE

Emergence and Spread of Epidemic Multidrug-Resistant *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the most common nosocomial pathogens worldwide. Although the emergence of multidrug-resistant (MDR) *P. aeruginosa* is a critical problem in medical practice, the key features involved in the emergence and spread of MDR *P. aeruginosa* remain unknown. This study utilized whole genome sequence (WGS) analyses to define the population structure of 185 *P. aeruginosa* clinical isolates from several countries. Of these 185 isolates, 136 were categorized into sequence type (ST) 235, one of the most common types worldwide. Phylogenetic analysis showed that these isolates fell within seven subclades. Each subclade harbors characteristic drug resistance genes and a characteristic genetic background confined to a geographic location, suggesting that clonal expansion following antibiotic exposure is the driving force in generating the population structure of MDR *P. aeruginosa*. WGS analyses also showed that the substitution rate was markedly higher in ST235 MDR *P. aeruginosa* than in other strains. Notably, almost all ST235 isolates harbor the specific type IV secretion system and very few or none harbor the CRISPR/CAS system. These findings may help explain the mechanism underlying the emergence and spread of ST235 *P. aeruginosa* as the predominant MDR lineage.

Key words: multidrug-resistance, *Pseudomonas aeruginosa*, whole genome sequence, population structure.

Introduction

Antimicrobial resistance (AMR) is a global concern, as it threatens the effective treatment of infectious diseases (http://www.who.int/antimicrobial-resistance/en/; last accessed November 27, 2017). *Pseudomonas aeruginosa* is a representative nosocomial pathogen showing AMR, and a major cause of death in patients with cystic fibrosis (Hoban et al. 2003; Rodriguez-Rojas et al. 2012). In addition to having intrinsic drug resistance mechanisms, this bacterium is able to acquire exogenous genes, resulting in the emergence of multidrug-resistant (MDR) strains of *P. aeruginosa*, which are resistant to carbapenems, aminoglycosides, and fluoroquinolones. As MDR *P. aeruginosa* has a major impact on medical practice (Viedma et al. 2009), knowledge of the mechanisms underlying multidrug-resistance to antibiotics and the epidemiology of MDR *P. aeruginosa* will be necessary to overcome infections with these bacteria.

Pseudomonas aeruginosa resistance to carbapenems and aminoglycosides was shown to be mediated by the acquisition of drug resistance genes (Hancock and Speert 2000; Livermore 2002), whereas resistance to fluoroquinolones is mediated by gene mutations (Chen and Lo 2003). Acquisition of genes encoding several families of β-lactamases has been found to contribute to resistance to carbapenems, such as IMP and VIM (Queenan and Bush 2007). Similarly, acquisition of exogenous genes encoding several types of aminoglycoside-modifying enzymes, such as AAC (Poole 2005) and 16S rRNA methylases (Wachino and Arakawa 2012), was shown to contribute to aminoglycoside resistance. In contrast, mutations in target genes, including those encoding DNA gyrase (*gyrA* or *gyrB*) and topoisomerase IV (*parC* and *parE*), were found to contribute to fluoroquinolone resistance (Chen and Lo 2003). Interestingly, drug resistance genes acquired by MDR *P. aeruginosa* differ markedly among communities in various countries (Viedma et al. 2009; Seok et al. 2011).

Epidemiological studies utilizing multilocus sequence typing (MLST; http://pubmlst.org/perl/bigsdb/bigsdb.pl?page=pubmlst_paeruginosa_isolates; last accessed November 27, 2017), a powerful molecular method for typing bacteria, can help understand and identify current MDR *P. aeruginosa* strains worldwide. These epidemiological studies showed that *P. aeruginosa* sequence type (ST) 235 is the predominant global clinical isolate (Viedma et al. 2009; Giske et al. 2006; Samuelsen et al. 2010; Lepsanovic et al. 2008; Empel et al. 2007; Cholley et al. 2011; van Belkum et al. 2015; Kos et al. 2015), with 88.3% of MDR *P. aeruginosa* isolates resistant to carbapenems, aminoglycosides, and fluoroquinolones being classified as ST235 (Kitao et al. 2012). Despite the importance of this subgroup, and despite the first ST235 *P. aeruginosa* being isolated in 1997, the mechanism underlying its persistence remains largely unknown.

To address the key traits responsible for its emergence and to identify the novel features of MDR *P. aeruginosa*, whole genome sequence (WGS) analysis was performed on *P. aeruginosa* strains isolated from hospital patients between 2001 and 2013 throughout Japan, as well as in other countries. In this study, MDR *P. aeruginosa* was defined according to the criteria of the Ministry of Health, Labour, and Welfare of Japan (Kirikae et al. 2008), because the definition of MDR *P. aeruginosa* varied among previous studies (Falagas et al. 2006).

Materials and Methods

Pseudomonas aeruginosa Isolates

MDR *P. aeruginosa* strains were collected based on the criteria specified by the Ministry of Health, Labour, and Welfare of Japan, including resistance to fluoroquinolones (minimum inhibitory concentration [MIC] $\geq$ 4 μg/ml), carbapenems (MIC $\geq$ 16 μg/ml), and amikacin (MIC $\geq$ 32 μg/ml; Kirikae et al. 2008). Several nonMDR *P. aeruginosa* strains were also included in this study (supplementary data 1, Supplementary Material online). Of the 185 clinical isolates evaluated by WGS analyses, 158 were from Japan, one from Thailand, three from Vietnam, seven from Nepal, and 15 from Poland. Also tested was the nonMDR *P. aeruginosa* strain PAO1 (USA). These isolates were grown on LB medium, and genomic DNA was purified using DNeasy Blood & Tissue kits (Qiagen). This study also included 26 *P. aeruginosa* strains registered in the database through 2012. Data including MLST and the genes or regions carried by each isolate are summarized in supplementary data 1 and 4, Supplementary Material online. In addition, 150 nonMDR *P. aeruginosa* isolates were screened to assess the frequency of ST235 *P. aeruginosa* among these nonMDR isolates.

WGS and Secondary Analyses

Nextera paired-end multiplex libraries of the isolates were generated and sequenced on a Genome Analyzer IIx (Illumina), according to the manufacturer's instructions, to generate 93-bp paired-end reads. More than 110-fold coverage was archived for each isolate. In some experiments, MiSeq (Illumina) was used to generate 251-bp paired end reads. The sequence data have been registered in the DNA Data Bank of Japan (DDBJ) under accession numbers DRA002419, DRA001216, and DRA001252. To identify SNPs among the isolates, all reads were assembled de novo into contigs using CLC Genomics Workbench (CLC bio), and the resulting contigs were concatenated by 60 × "*n*" gap filling and used as a reference genome. To identify SNPs among the 136 ST235 isolates, reads from each were aligned against the genome sequence of a reference ST235 strain, NCGM2.S1 (Miyoshi-Akiyama et al. 2011), excluding regions of prophages and integrons (supplementary table 1, Supplementary Material online), to obtain high-quality concatenated SNP sequences. The average quality of the SNPs identified was Qv35, covered by > 95% of the total reads.

MLST Analysis, O-type Analysis, and Analyses of Gene Distributions

MLST typing of all isolates was performed using the MLST plug-in of CLC Genomics Workbench with the MLST scheme for *P. aeruginosa* (Curran et al. 2004). The O-type of each O-type sequence was also determined using CLC Genomics Workbench. To analyze the distribution of drug resistance genes contributing to resistance against β-lactams, carbapenems, and aminoglycosides, as well as the distribution of genes contributing to *oprD* disruption and to the acquisition of exogenous genetic materials, Illumina reads of each isolate were assembled de novo and the resulting contigs were searched with the BLAST algorithm (Altschul et al. 1990) for each gene or region using CLC Genomics Workbench. The numbers of CIRSPRs and spacers were analyzed using CRISPRfinder (http://crispr.i2bc.paris-saclay.fr; last accessed

November 27, 2017; Grissa et al. 2007). Genes for CRISPR-associated proteins (CASs) harbored by *P. aeruginosa* retrieved from the NCBI database (supplementary table 2, Supplementary Material online) were used as queries to search for genes encoding CAS in *P. aeruginosa* strains used in this study. These results are presented in supplementary data 1, Supplementary Material online.

Phylogenetic Analyses

Concatenated SNP sequences were aligned with MAFFT (Katoh and Frith 2012). Evolutionary models (TVM + I + G for analysis of the 185 isolates and TVM + G for analysis of the 136 ST235 isolates) were chosen based on the results obtained with jModelTest 2.1.2 (Posada 2008) and convergence of the trees during preliminary phylogenetic analyses. Maximum-likelihood phylogenetic trees were constructed for the total 185 isolates and for the 136 ST235 isolates by concatenated SNPs with PhyML 3.0 (Guindon et al. 2010). The probability for node branching was evaluated with 100 bootstraps. In BEAST phylogeny of the 136 ST235 isolates, a clock model was chosen based on preliminary analyses showing better convergence of the tree. All other parameters were set to default, with chain lengths of 895,564,000 states and resampling every 10,000 states. Effective sample sizes (ESS) were >200 for all parameters. Time from the appearance of the most recent common ancestor was estimated using BEAST (Drummond and Rambaut 2007) and Path-O-Gen v1.4 (http://tree.bio.ed.ac.uk/software/pathogen/; last accessed November 27, 2017) programs.

Results

WGS analysis was performed on 156 MDR and 29 sensitive or nonMDR *P. aeruginosa* isolates (total 185 isolates) collected from patients throughout Japan and in other countries, including Thailand, Vietnam, Poland, and Nepal (supplementary data 1, Supplementary Material online). To determine the genetic relationships among these isolates, phylogenetic analysis was performed based on SNP concatenation. As the *P. aeruginosa* genome shows a high degree of plasticity, it is not sufficient to map Illumina reads to a particular *P. aeruginosa* genome. Thus, to determine sequence information on all of these isolates, the reads from all 185 libraries were assembled de novo, yielding 8,930 contigs (17,592,198 bp). These contigs were concatenated and used as the reference sequence to map reads from each isolate and to determine high-quality SNPs. A total of 249,067 SNPs were identified, and these concatenated SNP sequences were used to reconstruct a maximum-likelihood phylogenetic tree by MEGA5 (Tamura et al. 2011; fig. 1). One hundred thirty six of the 185 isolates (73.5%) were clustered into a ST235 clade, with 125 of these 136 isolates (80.1%) found to be MDR *P. aeruginosa*, consistent with findings of previous studies (Cholley et al. 2011; Kitao et al. 2012; Kirikae et al. 2008; Yoo et al. 2012), indicating that ST235 was the predominant clade in these *P. aeruginosa* isolates. Strikingly, screening of 150 nonMDR *P. aeruginosa* clinical isolates identified only two ST235 isolates, indicating that ST235 isolates are significantly enriched in the MDR *P. aeruginosa* population (chi-square test, $P < 0.001$).

Divergence within Subclades of ST235

As ST235 was the dominant MDR *P. aeruginosa* isolates, we analyzed the detailed relationships of the 136 ST235 isolates based on a Bayesian phylogenetic tree (Drummond and Rambaut 2007) and a maximum-likelihood phylogenetic tree constructed using the concatenated SNP sequence of these 136 ST235 isolates (fig. 2 and supplementary fig. 1, Supplementary Material online). The SNPs of each ST235 strain were compared with a representative ST235 MDR *P. aeruginosa* strain, NCGM2.S1 (Miyoshi-Akiyama et al. 2011). To determine the genetic backbone of these isolates, SNPs located in mobile elements, such as prophages and integrons (supplementary table 1, Supplementary Material online), were omitted from the concatenations. Phylogenetic analysis therefore included 34,926 SNPs. Bayesian phylogeny was thought to be more robust than maximum likelihood phylogeny, based on posterior probability (values for all subclades were >0.99) and bootstrap values, respectively. Therefore, each subclade was analyzed in detail based on Bayesian phylogeny.

The Bayesian phylogenetic tree resulted in the clustering of the 136 ST235 isolates into seven subclades (fig. 2). NP9, which was isolated from a patient in Nepal, was not included in any clade and was far separated from the other clades. Interestingly, NP9 was the only isolate expressing New Delhi metallo-beta-lactamase-1 (NDM1). The isolate from Thailand was clustered in subclade 5 and the isolates from Poland in subclades 4 and 7. None of the isolates from Vietnam was classified as ST235.

Assessments of the relationships between phylogenetic results and the geographic location of the isolates in Japan (fig. 2, and supplementary fig. 1, Supplementary Material online) showed that all isolates in subclade 3 were from one prefecture in Eastern Japan and most of the isolates in subclade 4 were from Western Japan. Six of the 11 isolates in subclade 7 were from Poland, with most of the remaining subclades being from Eastern Japan. The geographical distribution of isolates in each subclade suggests that the ancestors of each clade had spread on a global scale. Comparison of unique SNPs in each subclade with the genome of NCGM2.S1 (supplementary table 3 and supplementary data 2, Supplementary Material online) showed that subclades 3 and 4 had specific regions with unique SNPs; for example, 1,956,904_1,968,105 (NCGM2_1807 to NCGM2_1819) and 4,195,981_4,221,917 (NCGM2_3853 to NCGM2_3884) in subclades 3 and 5,270,138_5,299,359 (NCGM2_4888 to NCGM_4926) and 5,796,989_5,802,393 (NCGM2_5407 to

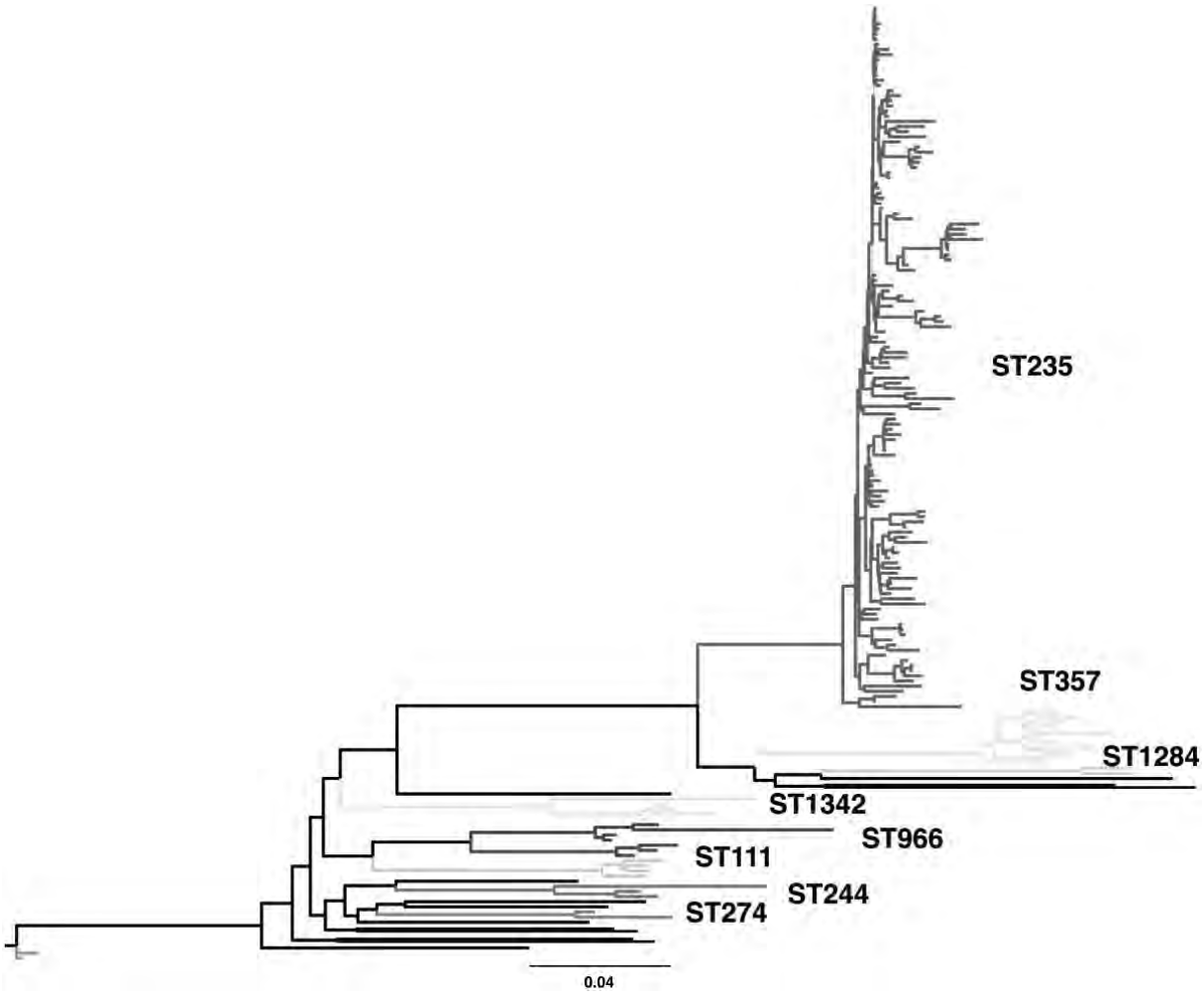

Fig. 1.—Phylogenic tree of the 185 *Pseudomonas aeruginosa* strains. The unrooted phylogeny of all *P. aeruginosa* strains was based on the maximum-likelihood method using PhyML 3.0 (Guindon et al. 2010). Each sequence type with more than one isolate [i.e. ST27 ($n = 2$), ST111 ($n = 3$), ST235 ($n = 136$), ST244 ($n = 3$), ST274 ($n = 2$), ST277 ($n = 5$), ST357 ($n = 11$), ST966 ($n = 4$), ST1284 ($n = 2$), and ST1342 ($n = 4$)] is indicated in color. Scale bar: 0.04 substitutions per variable site. Each main branch had >99% bootstrap support.

NCGM2_5412) in subclade 4. The geographic location and the accumulated unique SNPs in regions in each subclade, especially in subclades 3 and 4, suggest that each subclade evolved independently following its emergence in different communities after separation from their ancestors. Because some isolates obtained from countries other than Japan were closely related with subclades that included isolates from Japan such as subclades 4 and 5, the ancestors of these isolates were likely present in different countries independently.

More than 50% of the drug-resistance genes were conserved in multiple subclades (fig. 2, supplementary fig. 1 and supplementary data 1, Supplementary Material online). The genes *blaTEM, imp1 or 6*, *aac(6′)-Iae* and *aadA1* were conserved in subclades 1, 2, and 3; *aac(3)-Ic*, *aac(6′)-Ib*, *aacA4* and *aadA2* were conserved in subclades 5 and 6; and *imp7* was conserved in subclade 6. In addition, *oxa-2*, *aacA7*, *aacA8*, and *aadA6* were conserved in subclade 4, and *aac(6′)-Ib-cr*, *aacA4*, *aadA6*, and *aphA15* were conserved in subclade 7. These gene distribution patterns indicate that each subclade harbors specific drug resistance genes.

Subclades could also be distinguished by disruption of the *oprD* gene, which has been reported associated with carbapenem resistance (Lister 2002; Strateva and Yordanov 2009). In the prototype ST235 MDR strain, NCGM2.S1, which was clustered in subclade 1, *oprD* was found to be disrupted by insertion of an integron harboring *imp-1* and *aac(6′)-Iae*(Miyoshi-Akiyama et al. 2011). Disruption of *oprD* was highly prevalent in isolates

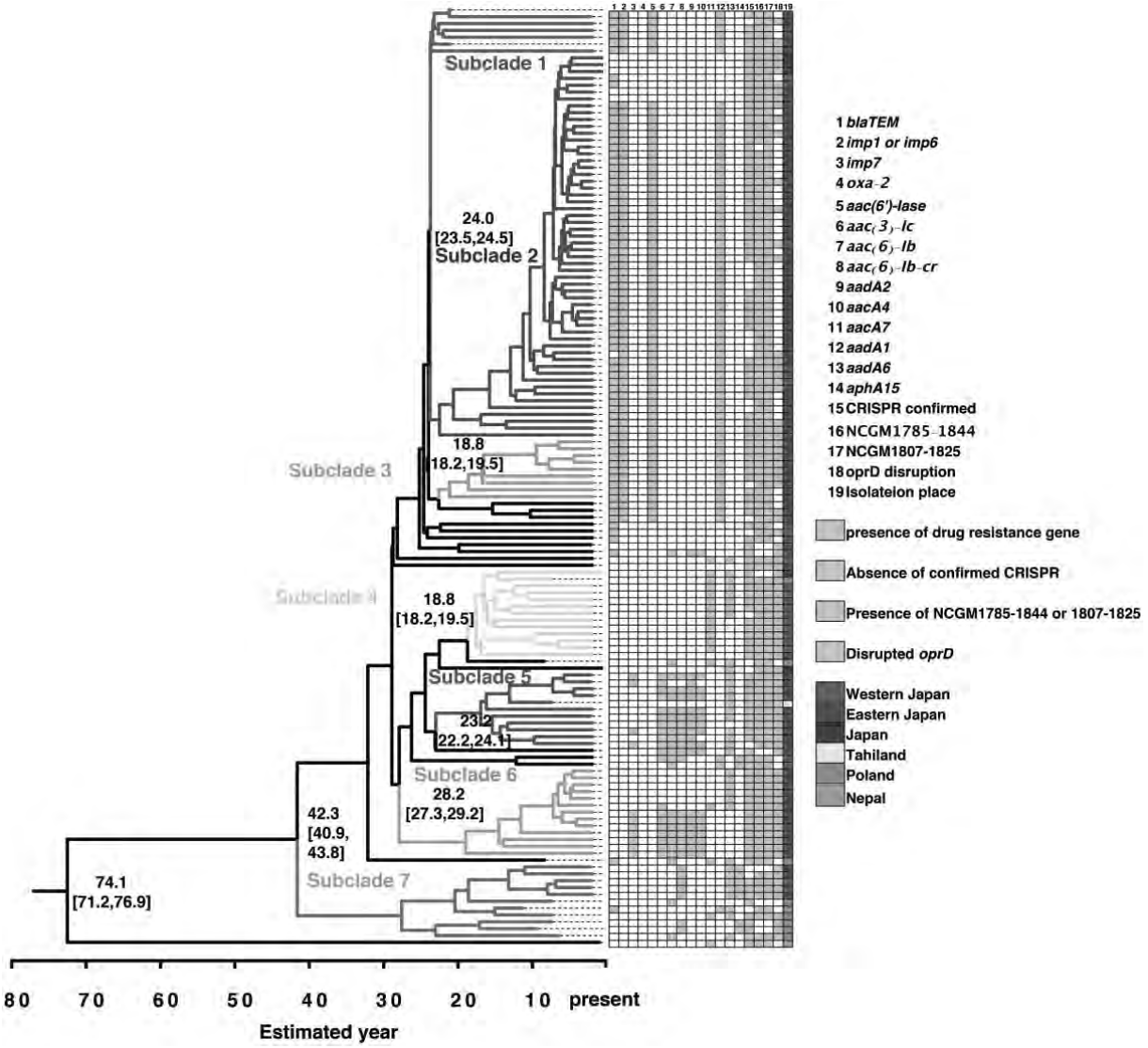

Fig. 2.—Phylogenic tree of the 136 ST235 *Pseudomonas aeruginosa* strains. The phylogeny of ST235 MDR *P. aeruginosa* strains was evaluated using the BEAST program (Drummond and Rambaut 2007). The seven subclades are shown in color. The estimated ages of the branches are shown as median values with 95% highest posterior density (HPD). The proportion of isolates from each location carrying conserved antibiotic resistance genes (>50% per subclade) and their *oprD* disruption status are also indicated. The posterior probability value for each main branch was >0.99. More detailed results are shown in supplementary figure 2 and supplementary data 1, Supplementary Material online. The posterior probability of each subclade designated was > 0.95.

of subclades 1 and 2, with 52 of 62 (83.9%) harboring *imp-1* and 55 of 62 (88.7%) harboring *aac(6′)-Iae*. The isolates in subclades 1 and 2 may have derived from a common ancestor containing an integron harboring *imp-1* and *aac(6′)-Iae* and spread throughout Eastern Japan.

Bayesian phylogenetic analysis also suggested that the most recent common ancestor of ST235 appeared ~74.1 (95% highest posterior density, 71.2–76.9) years ago and that branching within each subclade occurred ~20–40 years ago (fig. 2). Additionally, Path-O-Gen (http://tree.bio.ed.ac.uk/software/pathogen/; last accessed November 27, 2017), a tool for investigating the temporal signal and "clocklikeness" of molecular phylogenies, suggested that the most recent common ancestor of the 136 ST235 isolates emerged ~86.0 years ago. These results suggest that after emergence, the ancestors of these subclades were separated, with each acquiring particular drug resistance genes over the last 20–

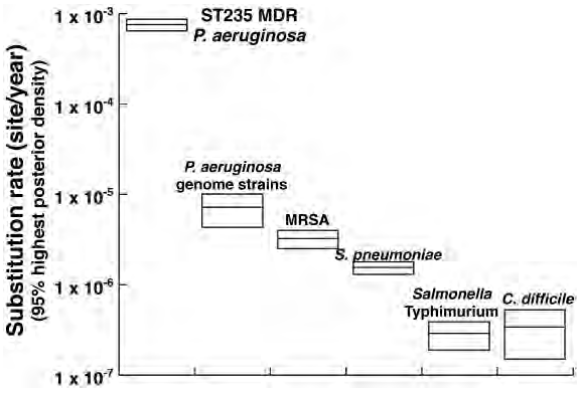

Fig. 3.—Substitution rate of various bacteria, including ST235 *Pseudomonas aeruginosa*. Comparison of reported substitution rates of MRSA (Harris et al. 2010), *Streptococcus pneumonia* (Croucher et al. 2011), *Salmonella typhimurium* (Okoro et al. 2012), *Clostridium difficile* (He et al. 2013), ST235 MDR *P. aeruginosa*, and *P. aeruginosa* (supplementary 4, Supplementary Material online). The substitution rates of *P. aeruginosa* were estimated using the BEAST program (Drummond and Rambaut 2007).

40 years, possibly coincident with the increased availability of antibiotics, such as aminoglycosides and carbapenems. The acquisition of drug resistance genes may have led to the expansion of each subclade over the past several decades.

Taken together, phylogenetic evidence suggests that several outbreaks accompanied the clonal expansion of ST235 MDR *P. aeruginosa* over the past several decades, and that these outbreaks may have been the driving force behind the generation of the population structure of ST235 MDR *P. aeruginosa* in Japan.

High Substitution Rate in ST235 MDR *P. aeruginosa*

Bayesian analyses suggested that the estimated substitution rate of ST235 MDR *P. aeruginosa* was between 6.44 and 8.66×10^{-4} (95% highest posterior density) substitutions per site per year (fig. 3). This substitution rate is at least 100-fold higher than those of other bacterial species (Harris et al. 2010; Croucher et al. 2011; Okoro et al. 2012; He et al. 2013). We analyzed the genome sequences of *P. aeruginosa* isolates, determined at least at the scaffold level registered in the database (https://www.ncbi.nlm.nih.gov hereafter referred to as "genome strains," supplementary table 4, Supplementary Material online) with the same parameters. The results showed a substitution rate of 4.3×10^{-6} to 1.0×10^{-5}, which was lower than that of ST235 MDR *P. aeruginosa* (fig. 3). These results suggested that the substitution rate of genomes in the ST235 *P. aeruginosa* isolates was relatively high.

Unique Features of ST235 MDR *P. aeruginosa*

To examine why ST235 isolates are dominant among MDR *P. aeruginosa*, we analyzed genes not having homologs in the representative nonMDR *P. aeruginosa* strains PAO1 and LESB58, but found uniquely in ST235 *P. aeruginosa*. Of the 827 genes found to be conserved in NCGM2.S1, but not in PAO1 or LESB58, 466 were present at frequencies >95% in the 136 ST235 *P. aeruginosa* strains (supplementary table 5 and supplementary data 3, Supplementary Material online). We identified five regions in ST235 isolates (NCGM2_1785 to NCGM2_1844, NCGM2_1949 to NCGM2_1963, NCGM2_3701 to NCGM2_3710, NCGM2_3761 to NCGM_3772, and NCGM2_5888 to NCGM2_5904) containing unique genes with high density. Although four of these regions contained genes that presumably contribute to metabolism or are annotated as hypothetical proteins, one region, NCGM2_1785 to NCGM2_1844, showed a high degree of similarity to a region previously identified as an ExoU island in *P. aeruginosa* 6077 (Kulasekara et al. 2006). This region encodes type IV secretion systems (T4SS), which have been extensively characterized and shown to mediate conjugation, uptake, and release of DNA, as well as effector translocation (Abajy et al. 2007; Alvarez-Martinez and Christie 2009; Wallden et al. 2010; Smillie et al. 2010; Stingl et al. 2010). The T4SS harbored by >95% of 136 ST235 strains analyzed in this study was the Tfc type (supplementary fig. 2 and table 6, Supplementary Material online, NCGM2.S1 was used as the representative), which contributes to conjugation (Mohd-Zain et al. 2004). PAO1 harbors nine genes or clusters, including competence-associated genes and T4SS, highly identical to genes in other *P. aeruginosa* strains (supplementary fig. 3 and supplementary data 4, Supplementary Material online). In contrast to PAO1, the additional T4SS found in ST235 isolates was not conserved among the *P. aeruginosa* strains, except for the ST235 strain 39016. Strains negative for the additional T4SS, such as PAO1 and LBSE58, did not harbor any drug resistance genes, suggesting that ST235 strains with additional T4SS are prone to acquisition of exogenous genetic material.

Absence of CRISPR/CAS System in ST235 *P. aeruginosa*

Additional genetic traits affecting the acquisition of exogenous genetic material have been reported to cluster in regularly-interspaced short palindromic repeats (CRISPRs) and in CASs (CRISPR/CAS; Bhaya et al. 2011; Wiedenheft et al. 2012). CRISPRs rely on small RNAs for sequence-specific detection and silencing of foreign nucleic acids, including bacteriophages and plasmids. Although the distributions of CRISPRs and CASs differed among the 26 *P. aeruginosa* genome strains, the ST235 isolates NCGM2.S1 and 39016 did not harbor more than one confirmed CRISPR and had relatively few CRISPR spacers (supplementary fig. 3 and supplementary data 4, Supplementary Material online). Furthermore, NCGM2.S1 and 39016 may not harbor recognizable CAS1 proteins. To confirm this tendency, the numbers of CRISPRs and spacers among the 136 ST235 *P. aeruginosa* strains were counted,

using CRISPRfinder (http://crispr.i2bc.paris-saclay.frlast; accessed November 27, 2017; Grissa et al. 2007). Remarkably, most ST235 isolates did not harbor confirmed CRISPRs and spacers, with the numbers of both varying in other ST isolates. We also analyzed whether the 136 ST235 strains harbored the 149 genes encoding CASs of *P. aeruginosa* listed in supplementary table 2, Supplementary Material online. The results indicated that none of the ST235 isolates harbored genes encoding CAS except for only 2 strains (PA1604, PL3220) (for PA1604: *csf1*, *csf2*, *csf3*, and *csf4*; for PL3220: *cas1 cas6/csy4*, and *csf2*; supplementary data 1, Supplementary Material online).

These results suggest that ST235 strains without the CRISPR/CAS system are prone to acquisition of exogenous genetic material.

Discussion

In this study, the features of MDR *P. aeruginosa* were determined by WGS analyses of 185 *P. aeruginosa* isolates isolated from hospitalized patients. MLST analysis showed that ST235 was the predominant clonal ST lineage, being identified in 136 of the 185 strains. These 136 strains, consisting of isolates from Japan and other countries, could be divided into seven subclades, each of which had specific features, including conserved drug-resistance genes, geographic location and genomic background. Estimates suggested that the most recent common ancestor of these ST235 isolates arouse ~80 years ago, with each subclade radiating over a few decades. These findings suggest that the ancestor of each subclade emerged over a few decades and spread in particular communities. WGS analysis also showed that ST235 MDR *P. aeruginosa* had a higher substitution rate, an additional T4SS, and no functional CRIPSR/Cas interference. These genetic traits may enable these bacteria to acquire exogenous genetic elements more efficiently, as well as increasing the intrinsic high competency of *P. aeruginosa*. Most currently used antibiotics were developed and used in patients from the 1960s to the 1980s. The acquisition of resistance to commonly used antibiotics, whether by substitution or the acquisition of drug resistance genes, may be the driving force behind the persistence and expansion of MDR ST235 *P. aeruginosa* in hospitals over the last several decades. Selection pressure from antibiotic treatment would allow the expansion of particular strains, such as ST235, that efficiently acquire substitution and drug resistance genes.

In conclusion, these results provide clues to the mechanism underlying the emergence and spread of MDR *P. aeruginosa* in particular communities and the key traits of the predominant ST235 MDR *P. aeruginosa* strain.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Author Contributions

T.M.A. designed the experiments and wrote the manuscript; T.T., N.O., and T.K. collected the *P. aeruginosa* isolates, contributed experimental suggestions, and strengthened the writing of the manuscript; N.V.H., P.T., B.M.P., and M.S. collected the *P. aeruginosa* isolates. All authors reviewed and provided comments to the text.

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Short Communication

Emergence of colistin-resistant *Escherichia coli* clinical isolates harboring *mcr-1* in Vietnam

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ABSTRACT

The *mcr-1* was first detected on a plasmid in colistin-resistant *Escherichia coli* from livestock and patients in China. We described here the emergence of colistin-resistant *E. coli* clinical isolates harboring *mcr-1* on the chromosomes in Vietnam. To our knowledge, this is the first report of hospital-acquired *E. coli* isolates harboring *mcr-1* in a medical setting in Vietnam.

The *mcr-1* gene was first reported to be a plasmid-encoded colistin resistance gene in *Escherichia coli* isolates from food animals and patients in China (Liu et al., 2016). Subsequently, *mcr-1*-positive strains have been found worldwide in several species of *Enterobacteriaceae* (Poirel et al., 2017). This report describes two colistin-resistant *E. coli* clinical isolates from Vietnam harboring chromosomal *mcr-1*.

A total of 18 multidrug-resistant *E. coli* isolates were obtained in a routine screening of multi-drug resistant Gram-negative pathogens, defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Magiorakos et al., 2012), from March to December 2014 in a hospital in Vietnam. Of them, 2 isolates were resistant to colistin. *E. coli* strains NCGM-EC88 and NCGM-EC89 were isolated from pus and urine samples of two inpatients in 2014. MICs of various antibiotics were determined using the microdilution method, according to the guidelines of the Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2015). DNAs were extracted from the isolates using DNeasy Blood & Tissue kits (QIAGEN, Tokyo, Japan) and the entire genomes were sequenced by MiSeq (Illumina, San Diego, CA) and analysed using CLC genomics workbench version 8.0 (CLC bio, Tokyo, Japan). Multilocus sequence typing (MLST) was performed using the MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). Pulsed-field gel electrophoresis (PFGE) and Southern hybridization were performed to determine whether *mcr-1* was located on plasmids or chromosomes, as previously described (Shrestha et al., 2015). A probe for *mcr-1* was amplified by PCR using the primer sets as previously described (Liu et al., 2016).

As shown in Table 1, the MICs of antibiotics for *E. coli* strains NCGM-EC88 and NCGM-EC89 showed that both isolates were resistant to ciprofloxacin and colistin, and that NCGM-EC-89 was also resistant to aminoglycosides, whereas both isolates were susceptible to carbapenems. The MICs of colistin for NCGM-EC88 and NCGM-EC89 were 4 and 4 mg/L, respectively. Both isolates harbored *mcr-1*. NCGM-EC88 harbored *aac(3)-IId*,*aph(3′)-Ia* and bla_{CMY-2}; and NCGM-EC89 harbored *rmtB*, *aph(3′)-IIa*, $bla_{CTX-M-55}$, and their MLSTs were ST410 and ST457, respectively.

PFGE, Southern blotting and hybridisation analyses revealed that *mcr-1* was located on the chromosomes of both NCGM-EC88 and NCGM-89. The genomic environments surrounding *mcr-1* in both isolates are shown in Figure 1. The genomic environment surrounding *mcr-1* in NCGM-EC88 (nt 5 to nt 3,249; GenBank

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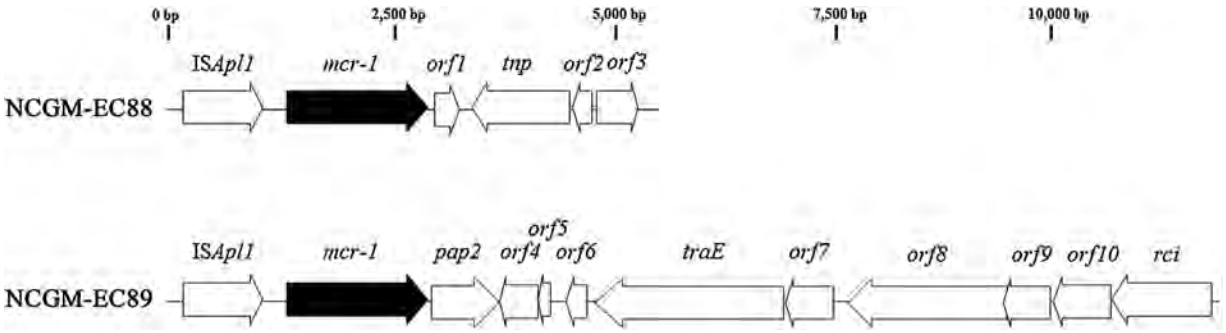

Figure 1. Genomic environments surrounding *mcr-1* in NCGM-EC88 and NCGM-EC89.

Table 1
MICs of various antibiotics for *E. coli* NCGM-EC88 and NCGM-EC89.

Antibiotics	MICs (μg/ml)	
	NCGM-EC88	NCGM-EC89
Amikacin	2	1,024
Arbekacin	≤0.5	>1,024
Ciprofloxacin	32	16
Colistin	4	4
Imipenem	≤0.5	≤0.5
Meropenem	≤0.5	≤0.5

accession no. LC193130) had a nucleotide sequence 99.6% identical to those in eight *E. coli* isolates, including six from food animals and two from humans. Of the six isolates from food animals, three, JS-B60 (GenBank accession no. KX254341), 59 (GenBank accession no. KX084394) and SHP45 (GenBank accession no. KU341381), were from the feces of pigs in China; EC2 (GenBank accession no. CP016184) was from the feces of a pig in Malaysia; S38 (GenBank accession no. KX129782) was from poultry meat in Switzerland, and RL465 (GenBank accession no. LT594504) was from a pig in Germany. Of the two isolates from humans, one, Af23 (GenBank accession no. KX032519), was from human blood in South Africa, and the second, ABC149 (GenBank accession no. KX013538), was from human blood in the United Arab Emirates. In seven of these isolates, *mcr-1* was encoded on a plasmid, whereas, in the eighth, *mcr-1* was encoded on a chromosome (GenBank accession no. LT594504).

The genomic environment surrounding *mcr-1* in NCGM-EC89 (nt 1 to nt 11,853; GenBank accession no. LC193131) had a nucleotide sequence 99.8% identical to those in three *E. coli* isolates, one from a food animal and two from humans; and to the sequence in one *Salmonella enterica* isolate, from a food animal. Of the isolates from animals, one *E. coli* SHP45 (GenBank accession no. KU341381), was from the feces of a pig in China, and the other, *Salmonella enterica* SC23 (GenBank accession no. KU934209), was from a chicken in China. Of the two isolates from humans, one, *E. coli* ABC149 (GenBank accession no. KX013538), was from human blood in the United Arab Emirates and the other, *E. coli* Af23, was from human blood in South Africa (GenBank accession no. KX032519).

The isolates NCGM-EC88 and NCGM-EC89 possibly come from food animals. These isolates belonged to ST410 and ST457, respectively, and several reports have described the isolation of *E. coli* ST410 and ST457 from food and pet animals in various countries around the world (Poirel et al., 2017). Each of these isolates, NCGM-EC88 and NCGM-89, possessed a single copy of an insertion element IS*Apl1* upstream of *mcr-1* (Figure 1), suggesting that IS*Apl1* could have contributed to the acquisition of *mcr-1* by these isolates. Moreover, *mcr-1* in *Enterobacteriaceae* might have been mobilized by a single upstream copy of IS*Apl1* (Snesrud et al., 2016), which also can contribute to the insertion of *mcr-1* from bacterial plasmids into chromosomes in NCGM-EC88 and NCGM-EC89.

Previously, a community-acquired clinical isolate of *Shigella sonnei* and animal-related *E. coli* isolates harboring *mcr-1*, the latter from food animals, have been reported in Vietnam (Malhotra-Kumar et al., 2016; Pham Thanh et al., 2016). To our knowledge, however, this is the first report of hospital-acquired *E. coli* isolates harboring *mcr-1* in a medical setting in Vietnam.

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RESEARCH ARTICLE Open Access

Dissemination of Carbapenem-resistant *Klebsiella pneumoniae* clinical isolates with various combinations of Carbapenemases (KPC-2, NDM-1, NDM-4, and OXA-48) and 16S rRNA Methylases (RmtB and RmtC) in Vietnam

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Abstract

Methods: Twenty-seven clinical isolates of carbapenem-resistant *Klebsiella pneumoniae* with MICs ≥4 mg/L for imipenem or meropenem were obtained from inpatients in a hospital in Vietnam. Antimicrobial susceptibility tests and whole genome sequencing were performed. Multilocus sequence typing and the presence of drug resistant genes were determined and a maximum-likelihood phylogenetic tree was constructed by SNP alignment of whole genome sequencing data.

Results: All the isolates harbored one of genes encoding carbapenemases, including KPC-2, NDM-1, NDM-4 and OXA-48. Of the isolates, 13 were resistant to arbekacin with MICs ≥256 mg/L and to amikacin with MICs ≥512 mg/L. These isolates harbored a gene encoding a 16S rRNA methylase, either RmtB or RmtC. Eighteen and 4 isolates belonged to international clones, ST15 and ST16, respectively. None of the isolates had colistin-resistant factors.

Conclusion: Carbapenem-resistant *K. pneumoniae* isolates belonged to international clones spread in a medical setting in Vietnam, and that these isolates harbored genes encoding various combinations of carbapenemases and 16S rRNA methylases. This is the first report of KPC-2, NDM-4 and OXA-48 producers in a medical setting in Vietnam.

Keywords: Carbapenem-resistant *Klebsiella pneumoniae*, Carbapenemase, Molecular epidemiology, MLST

Background

Emergence of carbapenemase-producing *Klebsiella pneumoniae* isolates has become serious problems worldwide [1]. These isolates produce several carbapenemases belonging to class A, B, and D, including KPCs, NDMs and OXA-48, respectively [2]. KPC-1 was initially found in a carbapenem-resistant strain *K. pneumoniae* 1534, which was collected in a surveillance during 1996 to 1997 in the United States hospitals [3]. NDM-1 was initially identified in *K. pneumoniae* and *Escherichia coli* in 2009 in Sweden [4]. Since then, NDM-1-producing *Enterobacteriaceae* have been reported worldwide [5]. OXA-48 was first identified in *K. pneumoniae* 11,978, which was isolated in 2001 in Turkey [6].

K. pneumoniae producing 16S rRNA methylase genes responsible for an extremely high level of resistance to various aminoglycosides have been increasingly reported [7]. To date, 10 types of 16S rRNA methylases, including

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ArmA, RmtA, RmtB, RmtC, RmtD, RmtE, RmtF, RmtG, RmtH and NpmA, have been found in clinical isolates. Of them, RmtB spread widely among various bacterial species, including *Acinetobacter baumannii*, *Enterobacteriaceae* and *Pseudomonas aeruginosa*, and RmtC spread among *Enterobacteriaceae* [7].

Methods

Bacterial strains and antimicrobial susceptibility

Twenty-seven *K. pneumoniae* isolates with minimum inhibitory concentrations (MICs) ≥4 mg/L for imipenem or meropenem were obtained from 27 inpatients treated at a hospital, Vietnam, from from February 2014 to April 2015. Of them, 22 isolates were from respiratory tracts, 3 from pus samples, 1 from a bile sample, and 1 from a urine sample. The isolates were phenotypically identified and species identification was confirmed by 16S rRNA sequencing. MICs were determined using the microdilution method, according to the guidelines of the Clinical Laboratory Standards Institute (M100-S25). The colistin MICs were also determined by Etest in colistin-resistant isolates evaluated by broth microdilution method.

Detection of antibiotic-resistance genes and their genetic environments

The entire genome of each isolate was extracted by DNeasy Blood & Tissue kit (QIAGEN, Tokyo, Japan) and sequenced by MiSeq (Illumina, San Diego, CA). Sequences of drug-resistance genes, including β-lactamase encoding genes at the website (https://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources/), aminoglycoside resistance genes (aminoglycoside-acetyltransferase, –adenylyltransferase and -phosphotransferase encoding genes), colistin resistance genes (*mcr-1*, *mcr-2* and *mgrB*), registered in GenBank (http://www.ncbi.nlm.nih.gov/nuccore/) and quinolone resistance genes *gyrA* and *parC*, were determined using CLC genomics workbench version 9.0.1. Genetic environments surrounding $bla_{\text{KPC-2}}$, bla_{NDMs} and $bla_{\text{OXA-48}}$ and the genes encoding the 16S rRNA methylases were determined.

MLST and phylogenetic analysis

Multilocus sequence types (MLSTs) were deduced as described in the protocols of the Institut Pasteur MLST (IP-MLST) (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html) databases. Clonal complexes (CC) were determined by eBURST version 3 (http://eburst.mlst.net). Single nucleotide polymorphisms (SNPs) of the genome sequences of all carbapenem-resistant isolates tested were identified by comparisons with the sequence of NDM-1 producing ST15 *K. pneumoniae* PMK1, (Gen Bank accession no. CP008929), with all the reads of each isolate aligned against the PMK1 sequence using CLC Genomic Workbench version 9.0.1. SNP concatenated sequences were aligned using MAFFT (http:/mafft.cbrc.jp/alignment/server/). Phylogenetic trees were constructed from the SNP concatemers. Models and parameters used for the phylogenetic analyses were computed using j-Model Test-2.1.4. A maximum-likelihood phylogenetic tree was constructed from SNP alignment with PhyML 3.0.

Pulsed-field gel electrophoresis and southern hybridization

The plasmids in each ST strain were extracted and pulsed-field gel electrophoresis was performed as describing previously [8]. Probes for $bla_{\text{KPC-2}}$, bla_{NDMs} and $bla_{\text{OXA-48}}$ were amplified by PCR using the primer sets as follows; KPC-F-TCGCTAAACTCGAACAGG and KPC-R-TTAC TGCCCGTTGACGCCCAATCC for $bla_{\text{KPC-2}}$, NDM-F- T TGGCCTTGCTGTCCTTG and NDM-R- ACACCAGTG ACAATATCACCG for bla_{NDMs} and OXA-48-F-TGTT TTTGGTGGCATCGAT and OXA-48-R-GTAAMRATGC TTGGTTCGC for $bla_{\text{OXA-48}}$, respectively. Signal detection was carried out using DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Applied Science, Indianapolis, IN).

Nucleotide sequence accession numbers

The whole genome sequences of all 27 isolates have been deposited at GenBank as accession numbers DRA005275.

Results

Antimicrobial susceptibility

MICs of 27 carbapenem-resistant isolates were shown in Table 1. All the isolates had MIC_{50} 8 mg/L and MIC_{90} 64 mg/L for imipenem, and MIC_{50} 8 mg/L and MIC_{90} 128 mg/L for meropenem. They were resistant to ampicillin with MICs ≥1024 mg/L, to aztreonam with MIC_{50} 512 mg/L and MIC_{90} 1024 mg/L, to ceftazidime with MIC_{50} 256 mg/L and MIC_{90} > 1024 mg/L, and to ciprofloxacin with MIC_{50} 256 mg/L and MIC_{90} 512 mg/L. Of all the isolates, 19 isolates (70%) were resistant to amikacin with MIC_{50} 1024 mg/L and MIC_{90} > 1024 mg/L. They had MIC_{50} 256 mg/L and MIC_{90} > 1024 mg/L for arbekacin, MIC_{50} 0.25 mg/L and MIC_{90} 32 mg/L to colistin, and MIC_{50} 2 mg/L and MIC_{90} 2 mg/L to tigecycline. The colistin MICs of *K. pneumoniae* isolates were significantly higher by the microdilution method than by Etest. MICs of colistin using Etest were from 0.75 to 2 mg/L (Table 1).

Drug resistant genes

All isolates tested had a carbapenemase encoding gene, such as $bla_{\text{KPC-2}}$, $bla_{\text{NDM-1}}$, $bla_{\text{NDM-4}}$ and $bla_{\text{OXA-48}}$; and the majority had a 16S rRNA methylase encoding gene, such as *rmtB* and *rmtC* (Table 1). Of the all isolates, 19 had $bla_{\text{OXA-48}}$, 5 had $bla_{\text{NDM-4}}$, 2 had $bla_{\text{NDM-1}}$, and 1

Table 1 Summary of the characteristics of the 27 *Klebsiella pneumoniae* strains, including antimicrobial resistance profiles, resistance genes and MLST

Strain	MIC(mg/L)										Carbapenemases	Size of the plasmids harboring carbapenemase encoding genes	ESBL	16S rRNA methylases	Aminoglycoside modification enzymes	Mutations in DNA gyrase		MLST
	ABK	AMK	AMP	AZT	CAZ	CIP	CST[a]	IPM	MEM	TGC						*gyrA*	*parC*	
VNC Kp05	1024	>1024	>1024	512	256	256	4 (2)	8	8	1	OXA-48	50 kbp	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp10	256	512	>1024	1024	512	512	32 (2)	16	4	2	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	RmtB	-	S83Y, D87A	S80I	15
VNC Kp13	256	1024	>1024	512	256	256	32 (2)	8	8	2	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp16	1024	>1024	>1024	512	256	512	32 (2)	8	4	2	OXA-48	50 kbp	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp17	512	>1024	>1024	512	128	256	4 (2)	8	8	4	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp20	512	>1024	>1024	256	128	256	0.25	2	8	2	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp21	256	1024	>1024	512	128	256	0.25	2	4	2	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp22	1024	1024	>1024	512	256	256	16 (2)	16	16	2	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp25	256	>1024	>1024	256	256	256	0.125	8	2	2	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp28	512	1024	>1024	512	256	256	8 (0.75)	8	8	2	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp29	1	4	>1024	512	32	32	0.125	4	4	1	KPC-2	150 kbp	CTX-M-15, TEM-1, SHV-28	-	AAC(6′)-Ib-cr	S83I	S80I	307
VNC Kp30	1	2	>1024	512	128	128	0.25	4	4	2	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	-	AAC(6′)-Ib-cr, AADA16 APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp32	512	1024	>1024	1024	256	256	8 (1)	16	8	2	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp34	256	1024	>1024	512	256	256	32 (2)	8	8	1	OXA-48	ND	CTX-M-15, SHV-28, TEM-1	RmtB	AAC(6′)-Ib-cr, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp35	256	128	>1024	512	1024	256	16 (1)	8	8	2	OXA-48	ND	CTX-M-14, SHV-12, TEM-1	RmtB	AAC(6′)-Ib-cr, AADA1, APH(3″)-Ia	S83Y, D87A	S80I	15
VNC Kp39	>1024	>1024	>1024	32	>1024	256	0.5	8	8	1	NDM-1	100 kbp	CTX-M-27, SHV-28	RmtC	AAC(6′)-Ib-cr, AADA16	S83I	S80I	395
VNC Kp42	16	16	>1024	256	>1024	64	32 (1)	32	64	1	NDM-4	120 kbp	CTX-M-15, SHV-28, TEM-1	-	AAC(6′)-Ib-cr, AADA1, AADA16	S83F, D87A	S80I	15
VNC Kp43	1024	1024	>1024	512	256	256	0.25	4	4	2	OXA-48	ND	CTX-M-14, SHV-12, TEM-1	RmtB	AAC(6′)-Ib-cr, AADA1	S83Y, D87A	S80I	15
VNC Kp51	16	16	>1024	1024	256	64	0.25	16	4	1	OXA-48	ND	CTX-M-14, SHV-12, TEM-1	-	AAC(6′)-Ib-cr, AADA1	S83Y, D87A	S80I	15
VNC Kp54	>1024	>1024	>1024	1024	256	64	0.5	8	16	1	OXA-48	145.5 kbp	CTX-M-14, CTX-M-15, SHV-1, TEM-1	RmtB	AADA2, APH(3″)-Ia	S83F, D87N	E84K	16
VNC Kp56	>1024	>1024	>1024	512	256	64	0.25	16	16	1	OXA-48	ND	CTX-M-14, CTX-M-15, SHV-1, TEM-1	RmtB	AADA2, APH(3″)-Ia	S83F, D87N	E84K	16
VNC Kp57	>1024	>1024	>1024	64	>1024	256	0.25	64	128	0.5	NDM-4	40 kbp	CTX-M-27, SHV-11, TEM-1	RmtB	-	S83I	S80I	2353
VNC Kp68	2	4	>1024	512	256	64	0.125	4	8	2	OXA-48	50 kbp	SHV-1, TEM-1	-	AAC(6′)-Ib-cr	S83I	S80I	147
VNC Kp70	16	16	>1024	1024	>1024	256	0.125	128	256	2	NDM-4	120 kbp	CTX-M-14, CTX-M-15, SHV-1, TEM-1	-	AAC(6′)-Ib-cr, AADA1, AADA2	S83F, D87N	E84K	16
VNC Kp72	16	8	>1024	128	>1024	128	0.125	32	64	2	NDM-4	ND	CTX-M-15, SHV-28, TEM-1	-	AAC(6′)-Ib-cr, AADA1, AADA16	S83F, D87A	S80I	15
VNC Kp73	16	16	>1024	1024	>1024	512	0.125	128	256	2	NDM-4	ND	CTX-M-14, CTX-M-15, SHV-1, TEM-1	-	AAC(6′)-Ib-cr, AADA1, AADA2	S83F, D87N	E84K	16
VNC Kp77	>1024	>1024	>1024	128	>1024	256	0.25	16	64	1	NDM-1	ND	CTX-M-27, SHV-28	RmtC	AAC(6′)-Ib-cr, AADA16	S83I	S80I	395

MIC, minimum inhibitory concentration, *ABK* arbekacin, *AMK* amikacin, *AMP* ampicillin, *AZT* azidothymidine, *CAZ* ceftazidime, *CIP* ciprofloxacin, *CST* colistin, *IPM* imipenem, *MEM* meropenem, *TGC* tigecycline, *ESBL* extended-spectrum-lactamase, *ND* not determined

[a]MICs for colistin using Etest are given in parentheses

had bla_{KPC-2}. Seventeen had *rmtB* and 2 had *rmtC*. These isolates also had (an) extended spectrum β-lactamase encoding gene(s), including $bla_{CTX-M-14}$, $bla_{CTX-M-15}$, $bla_{CTX-M-27}$, bla_{SHV-1}, bla_{SHV-11}, bla_{SHV-12}, bla_{SHV-28}, bla_{SHV-55}, and/or bla_{TEM-1}; and aminoglycoside modification enzymes, including *aac(6′)-Ib-cr* and/or *aadA1* (Table 1). All isolates had 2 or 3 point mutations in the quinolone-resistance-determining regions of *gyrA* and *parC* (Table 1). None of the isolates harbor *mcr-1* or *mcr-2*, and our analysis did not reveal any isolates with disruption in the *mrgB* gene.

Genetic environments surrounding genes encoding carbapenemases

The genetic structure surrounding bla_{KPC-2}, bla_{NDM-1}, bla_{NDM-4} and bla_{OXA-48} were shown in Fig. 1. The genomic structure surrounding bla_{KPC-2} was identical with that of *Aeromonas hydrophila* strain WCHAH01 plasmid pKPC2 (GenBank accession no. KR014106), which was isolated in China.

All isolates harboring bla_{NDM-1} tested had the same genetic structure surrounding bla_{NDM-1} (Fig. 1), which was identical with that of plasmid pRIH26 in NDM-1 producing *K. pneumoniae* isolated from a patient in 2012 in Rhode Island, the United States [9]. This patient had returned to the United States after a hospitalization in Vietnam [9].

All isolates harboring bla_{NDM-4} tested had the same genetic structure surrounding bla_{NDM-4} (Fig. 1), which was identical to that of NDM-1 producing *K. pneumoniae* strain KP4 plasmid pKP04NDM isolated in China (GenBank accession no. KU314941).

OXA-48 producers had either one of two genetic structures surrounding bla_{OXA-48} (Fig. 1). Of them, one was not reported (the second structure from the bottom in Fig. 1), whereas the other was identical with plasmids in OXA-48 producing *K. pneumoniae* strains 153,877–1 in Netherlands (GenBank accession no. KP659188), KP112 in France (GenBank accession no. LN864819), Kpn-E1.Nr7 in Switzerland (GenBank accession no. KM406491), E71T in Ireland (GenBank accession no. KC335143), KP1 and KP2 in France (GenBank accession no. KC757416 and KC757417, respectively), and 23 plasmid pIncL_M_DHQP1400954 in the USA (GenBank accession no CP016927).

The bla_{KPC-2}, bla_{NDM-1}, bla_{NDM-4} and bla_{OXA-48} in each ST strain will be all located on plasmids and the sizes of the plasmids were shown in Table 1.

MLST and molecular phylogenetic analysis

The clinical isolates of *K. pneumoniae* tested belonged to either one of ST15, ST16, ST147, ST307, ST395 and ST2353 (Table 1). Of these isolates, 18 belonged to ST15 and 4 belonged to ST16 (Table 1). The new sequence

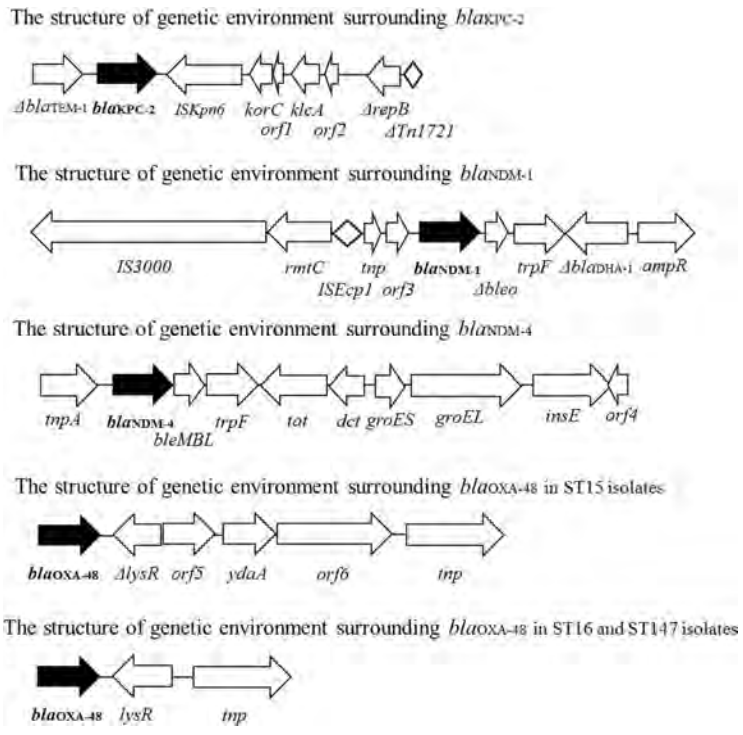

Fig. 1 The structure of genetic environments surrounding bla_{KPC-2}, bla_{NDM-1}, bla_{NDM-4} and bla_{OXA-48}

type, ST2353, belonged to CC147. Phylogenetic analysis revealed that the isolates belonging to ST15 formed the largest clade among the 27 isolates (Fig. 2a). The 18 isolates belonging to ST15 harbored either one of two genes encoding carbapenemases, including $bla_{\text{OXA-48}}$ and $bla_{\text{NDM-4}}$; and genes encoding aminoglycoside resistance factors, including *aac(6′)-Ib-cr* and *rmtB* (Table 1). The phylogenetic tree among 18 ST15 isolates showed two subclades, A and B (Fig. 1b). Of them, 16 belonging to subclade A harbored $bla_{\text{OXA-48}}$ and 2 belonging to subclade B harbored $bla_{\text{NDM-4}}$ (Fig. 2b).

Discussion

To our knowledge, this is the first report of the whole genome based molecular epidemiological analysis of carbapenem-resistant *K. pneumoniae* in Vietnam. Our study suggests that carbapenem-producing ST15 *K. pneumoniae* have been spreading in medical settings in Vietnam. A NDM-1 producing *K. pneumoniae* clinical isolate in Vietnam was firstly obtained from a urinary tract of a 62-year-old man in 2010 [10].

This is the first report of NDM-4 or OXA-48 producing *K. pneumoniae* in Vietnam. NDM-4 was firstly detected in *E. coli* I5, which was recovered from a urine sample of a patient hospitalized in 2010 in India [11]. Since then, NDM-4 producers were reported in *Enterobacter cloacae* in Sri Lanka [12], *E. coli* in India [13], Italy [14] and Vietnam [15], and *K. pneumoniae* in Japan [16]. NDM-4 possessed increased hydrolytic activity toward carbapenems and several cephalosporins compared to NDM-1 [11]. NDM-4 with an amino acid substitution at position 130 (Met to Leu) showed increased hydrolytic activity toward carbapenems and several cephalosporins compared to NDM-1 [11].

ST15 will be an emerging high-risk multidrug-resistant clone with carbapenem-encoding genes, including bla_{KPCs}, bla_{NDMs} and bla_{OXAs}. Outbreaks caused by ST15 OXA-48 produces were reported in France and Spain [2]. When Diancourt et al. [17] developed a MLST for *K. pneumoniae*

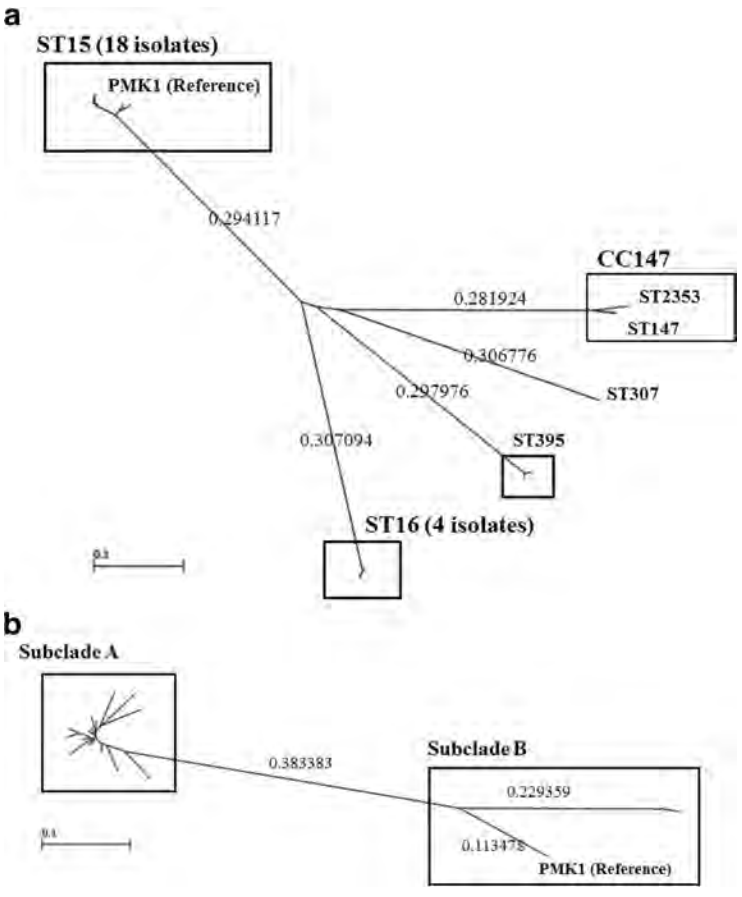

Fig. 2 **a** Molecular phylogeny of the 27 *K. pneumoniae* strains. A maximum-likelihood phylogenetic tree was constructed from the 27 carbapenem-resistant isolates. The isolates belonging to ST15 formed the largest clade among the 27 isolates. **b** Molecular phylogeny of the 18 *K. pneumoniae* strains belonging to ST15. A maximum-likelihood phylogenetic tree was constructed from the 18 carbapenem-resistant isolates. Of them, 16 belonging to subclade A harbored $bla_{\text{OXA-48}}$ and 2 belonging to subclade B harbored $bla_{\text{NDM-4}}$

in 2005, they already detected ST15 isolates from several countries in Europe, such as Austria, France, Portugal and Poland, and the most of the isolates were resistant to ceftazidime and ciprofloxacin. ST15 *K. pneumoniae* isolates were reported to spread in medical settings in 2005 in Hungary [2]. ST15 *K. pneumoniae* isolates were detected in other European countries, including Bulgaria, Croatia, Czech Republic, Denmark, Hungary, Italy, Netherlands, and Spain [2]; they were also detected in Asian countries, including China, South Korea, Malaysia, Singapore, Thailand, and Vietnam [2]; in African countries, including Côte d'Ivoire, Madagascar, Morocco, and Senegal [18]. These ST15 isolates frequently produced ESBL, including CTX-M, SHV-28 and TEM variants [19], and moreover, they became to produce various carbapenemases, including KPCs, OXA-48, NDMs and VIM-4 [19]. One of the well-recognized high-risk clones is CC258 which is frequently associated with KPCs-producing *K. pneumoniae* known as a high-risk clone [19], and these isolates were reported many countries, such as the United States, Greek, Norway, Sweden, Italy, Poland, Canada, Brazil and Korea [19]. ST11, a related clone ST258, was reported in KPCs-producing isolates mainly in China, but also in NDMs-producers from Czech Republic, Switzerland, Thailand, Australia, the United States, the United Arab Emirates and Greece [19].

Etest seems a more reliable method to measure colistin MICs than broth microdilution method [20, 21]. In the present study, 11 isolates were resistant to colistin with MICs 4–32 mg/L by broth microdilution method, although none of the isolates had colistin-resistant factors. Our previous study indicated that *Enterobacteriacae* isolates showed lower colistin MICs by Etest than by broth microdilution method [20]. It is necessary to find feasible susceptibility testing methods of determining the MICs of polymyxins for clinical laboratories.

Conclusions

This study showed that carbapenem-resistant *K. pneumoniae* isolates belonged to international clones spread, and that these isolates harbored genes encoding various combinations of carbapenemases and 16S rRNA methylases, in a medical setting in Vietnam.

Abbreviations

ESBLs: extended-spectrum β-lactamases; MBLs: Metallo-β-lactamases; MICs: minimal inhibition concentration; MLST: Multilocus sequence typing

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Biosafety Committee of the National Center for Global Health and Medicine (approval number: 27-M-52).

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Availability of data and materials

Nucleotide sequence accession numbers of the whole genome sequences of all 27 isolates have been deposited at GenBank as accession numbers DRA005275.

Authors' contributions

TT and MT: Performed whole genome sequencing, analyzed data and drafted the manuscript. KS: Performed drug-susceptibility tests. TTTN: Performed clinical bacterial analyses. LTAT and TTP: Designed protocols and supervised this study at CRH. NO and TK: Designed protocols and supervised this study. All authors read and approved the final manuscript.

Ethical approval and consent to participate

The study protocol was carefully reviewed and approved by the ethics committee of Cho Ray Hospital (approval number: 1644/QD-BVCR), the ethics committee of the National Center for Global Health and Medicine (No. 1268), respectively. Respectively. Individual informed consent was waived by the ethics committee listed above because this study used currently existing sample collected during the course of routine medical care and did not pose any additional risks to the patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

Publisher's Note

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IV. Appendix

1. List of Participants from Bach Mai Hospital Attending NCGM Training Courses

No	Name	Position	Department	Training course	Duration (days)	Date
1	Dr. Nguyen Duc Anh	Doctor	Neurosurgery	Project to Support Improvement of the Quality of Stroke Care in the Socialist Republic of Viet Nam - Introduction of a Comprehensive Team Care for Stroke	35	24/9-28/10
2	Mr. Dao Van Hieu	Nurse	Neurosurgery		14	24/9-7/10
3	Dr. Do Dao Vu	Dept. Deputy Head	Rehabilitation		14	24/9-7/10
4	Ms. Tran Lan Phuong	Physical thepapist	Rehabilitation		14	24/9-7/10
5	Ms. Nguyen Thi Phuong	Speech therapist	Rehabilitation		14	24/9-7/10
6	Dr. Dao Thi Thu Hoai	Head of Consultant unit	Nutrition Consultance, Nutrition Center		14	24/9-7/10
7	Pharm. Bui Thi Ngoc Thuc	Pharmcist	Pharmacy		14	24/9-7/10
8	Dr. Nguyen Cong Tan	Doctor	ICU	Strengthening clinical capacity of the perioperative medical care	14	16/10-27/10
9	Ms. Hoang Minh Hoan	Nurse	ICU		14	16/10-27/10
10	Dr. Tran Duc Minh	Doctor	Anesthesiology		14	16/10-27/10
11	Mr. Nguyen Sy Trang	Nurse	Anesthesiology		14	16/10-27/10
12	Mr. Nguyen Hoang Van	Dept. Deputy Head	Medical Equipment	Current state of medical equipment management in Viet Nam and countermeasure research and technology transfer	7	23/10-28/10
13	Dr. Ngo Gia Khanh	Doctor	Surgery	Promotional programs of general thoracic surgery	7	26/11-9/12
14	Ms. Nguyen Thi Thu Ha	Head	Quality Management of Heart Institute	Quality Management and Patient Safety	14	18/6-1/7

2. List of Participants from Other Hospitals Attending NCGM Training Courses

No	Name	Position	Department	Hospital	Training course	Duration (days)	Date
1	Dr. Uong Thanh Tung	Dept. Head	Quality Management	Saint Paul	Quality Management and Patient Safety	14	18/6-1/7
2	Dr. Do Thi Thu Hien	Dept. Acting Head	Outpatient	Hanoi Obstetrics and Gynecology Hospital		14	18/6-1/7
3	Dr. Pham Van Son	Hospoital Vice Director		Vinh City General Hospital		14	18/6-1/7
4	Dr. Thai Thi Thanh Thuy	Dept. Deputy Head	Quality Management	Da Nang Hospital for Women and Children		14	18/6-1/7
5	Dr. Phan Thi Hang	Hospital Vice Director		Hung Vuong Hospital		14	18/6-1/7
6	Dr. Vu Duy Tung	Dept. Head	Quality Management	Ba Ria Hospital		14	18/6-1/7
7	Mr. Phung Dac Thanh	Dept. Deputy Head	Nursing Department	Vinh Phuc Provincial General Hospital	Strengthening Ability for Clinical Instructor of Nursing	12	26/9-7/10
8	Ms. Nguyen Thi Kim Minh	Dept. Deputy Head	Nursing Department	Dong Nai Provincial General Hospital		12	26/9-7/10
9	Ms. Nguyen Thi Phuong Dinh	Dept. Deputy Head	Nursing Department	Saint Pault Hospital		12	26/9-7/10
10	Ms. Vu Thi Hong Nhung	Dept. Head Nurse	Pediatric Department	Dien Bien Provincial General Hospital		12	26/9-7/10
11	Dr. Lê Hải Sơn	Doctor	Surgery	108 Hospital		14	6/11-9/12

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