

*e-ASIA Joint Research Program  
Progress Report*

1. Project Title: Characterization of multidrug resistance Gram-negative bacteria carrying antibiotics-resistance gene which have been inserted to chromosome.

2. Joint Research Period: August 1st, 2016 ~ March 31th, 2020

3. Principal Investigators:

- Japan: Itaru Hirai, Ph.D, Professor, School of Health Sciences, Faculty of Medicine, University of the Ryukyus
  - Planned Funding Period: August 1st, 2016 – March 31th, 2020
- Vietnam: Bui Thi Mai Huong, MD, Department Head, Department of Food microbiology and Molecular Biology, National Institute of Nutrition.
  - Planned Funding Period: August 1st, 2016 – March 31th, 2020
- Indonesia : Kuntaman, MD, Ph.D, Professor, Department of Microbiology, Faculty of Medicine Airlangga University / Dr. Soetomo Academic Hospital Surabaya
  - Planned Funding Period: August 1st, 2016 – March 31th, 2020

4. Summary of the Progress of the Joint Research:

Detection rates of antimicrobial resistance (AMR) bacteria such as extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae have been rapidly increasing in many countries, especially in the South and Southeast Asian countries. Distribution of AMR bacteria is not limited within clinics, and have already been extent into many parts. For instance, CTX-M type ESBL-producing *Escherichia coli* has been observed in clinical specimens, community, environment, animals, food items and so forth. Certain AMR genes such as *bla*<sub>CTX-M</sub> and plasmid colistin resistant *mcr-I* have been mobilized by transposases, such as IS (insertion sequence) among transferrable plasmid and between the plasmid to chromosome. Many studies including ours reported that AMR bacteria isolates possessed chromosomally-located antimicrobial resistance gene. In addition, in this project, Prof. Shirakawa's group (Japan) analyzed clinical isolates obtained in Indonesia by collaborating with Indonesia group and showed 47.4% of the examined 57 strains harboring *bla*<sub>CTX-M-15</sub> possessed chromosomally-located *bla*<sub>CTX-M</sub> (submitted).

In these years, genetic information has been acquired by using the next generation sequencer. However, mostly due to technical limitation, it is not easy to determine location of such mobile antimicrobial resistance genes. Therefore, several laborious analytical methods are utilized to identify chromosomally-located antimicrobial genes. In order to establish and evaluate analytical methods for chromosomally-located antimicrobial resistance genes, we produced an experimental *Escherichia coli* strain which possessed two plasmids, *i.e.*, a plasmid harbored *bla*<sub>CTX-M-14</sub> transposition unit and a plasmid harbored genes for the CRISPR/Cas9 system. Consequently, we have established protocols for identification of chromosomally-located antimicrobial resistance genes in which included restriction digestion, inverse PCR, adaptor ligation and sequencing. By the established methods, it was indicated as follows, i) chromosomally-located *bla*<sub>CTX-M-14</sub> was randomly inserted to chromosome, ii) upstream genetic structure of chromosomally-located *bla*<sub>CTX-M-14</sub> was homologous but downstream genetic structure was not, iii) overall transfer frequency of *bla*<sub>CTX-M-14</sub> transposition unit in the experimental *E. coli* strain was about 0.5% (Prof. Hirai's group). At present, many AMR bacteria isolates from Vietnamese community, Indonesian community, Indonesian clinic and Japanese community are going to analyze by the methods. In addition, for improvement of the established methods and for high throughput determination of chromosomally-located antimicrobial resistance genes, DNA barcoding and Nanopore sequencer (Oxford Nanopore Technologies) are

going to be utilized.

As mentioned above AMR bacteria has been distributed even in community of many countries. Preliminary investigation by Indonesia, Vietnam and Japan groups indicated prevalence of ESBL-producing Enterobacteriaceae is clearly high in university students who were before clinical practice and healthy individuals in each community. In addition, preliminary investigation by Vietnam and Japan collaboration indicated certain intervention effectively had worked in a Vietnamese community. If the project would found out key target for intervention, there would some possibility to contain AMR bacterial distribution especially in community. Therefore field investigation regarding to AMR bacteria is important to grab current situation of AMR bacteria in community and to screen out potential key target for interventions. Until now ethical clearance was obtained and questionnaires have been prepared. Field investigations in Indonesian and Vietnamese communities are scheduled in year 2018.

5. Scientific Achievements and Implemented Activities (Research Exchange, Workshop, Publication, etc. if any):

*\*For this item, please fill in the attached Excel file.*

6. Future Goals and Plan of Activities within and after the project period:

As in our research proposal, AMR has been one of the biggest issues in almost every countries. Prevalence of AMR bacteria and its characterization has been performed by conventional molecular microbiological methods. Currently, next generation sequencing is also utilized to do so. Except for the PacBio system (Tomy Digital Biology Co. Ltd.) and Nanopore sequencer (Oxford Nanopore Technologies), identification of chromosomally-located antimicrobial resistance genes is not easily achieved even by using the next generation sequencer(s). Therefore, information regarding to chromosomally-located antimicrobial resistance genes is hardly accumulating. This project is utilizing the Nanopore sequencer to analyze chromosomally-located antimicrobial resistance genes within the project period. By using the Nanopore sequencer, this project is working on establishing analytical protocol of chromosomally-located antimicrobial resistance genes and its database.

Clinically and molecular epidemiologically, “raison d’etre” of the chromosomally-located antimicrobial resistance genes is not clear enough. Therefore, establishing and maintaining surveillance system(s) of AMR bacteria is necessary. Especially, surveillance system which includes analysis of antimicrobial resistance gene location is being craved. This project is working on to provide essential information for establishing the surveillance system for the chromosomally-located antimicrobial resistance genes.

Intervention can be one of effective cure for already well distributed AMR bacteria especially in community. To make effective intervention, background information surrounding community-distributed AMR bacteria and its contributing risk factor(s) are to be investigated. Therefore, field investigation with precise interview form(s) regarding AMR bacteria and its risk factors is planned in communities in Vietnam and Indonesia. The project is studying risk factor(s) for community-distributed AMR bacteria in order to perform effective intervention for AMR bacteria and to provide useful information regarding to AMR bacteria and chromosomally-located antimicrobial resistance genes for devising public health policy even after the project period.

7. Recommendations and Comments to the Program (if any):

(ex. Any support to request from the Program in order to achieve item 6.)

# Lists of Achievements and Implemented Activities

## 1. Original Publication of Articles etc.

### 【Notes】

Please fill in **only the achievements of this project** by country in order of publication date. Only “published” is targeted, but please write “in press” too only for Final Report.

Please count Proceedings with peer review as original paper.

The information on this form is only disclosable. Please submit Non-disclosable information in a separate file.

### 1. 1 Original Publications (Articles co-authored among Research Teams)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)
Taro Kagaku and Jiro Kagaku, Distinct roles of MLCK and ROCK in the regulation of membrane	doi: 10.1083/jcb.201506	in press	
Bagus Wasito E, Shigemura K, Osawa K, Fardah A, Kanaida A, Raharjo D, Kuntaman K, Hadi U, Harijono S, Marto Sudarmo S, Nakamura T, Shibayama K, Fujisawa M, Shirakawa T. Antibiotic Susceptibilities and Genetic Characteristics of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli Isolates from Stools of Pediatric Diarrhea Patients in Surabaya, Indonesia. Jpn J Infect Dis.70(4), 378–382, 2017.	doi: 10.7883/yoken.JJID.2016.234.	published	
Bui TK, Bui TM, Ueda S, Le DT, Yamamoto Y, Hirai I: Potential Transmission Opportunity of CTX-M-producing Escherichia coli in Large-scale Chicken Farm in Vietnam. J Glob Antimicrob Resist. 13, 1–6, 2017	doi: 10.1016/j.jgar.2017.09.014.	published	

2 Total

### 1. 2 Original Publications (Articles by Single Team only)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)	Country name of the team
Taro Kagaku and Jiro Kagaku, Distinct roles of MLCK and ROCK in the regulation of membrane	doi: 10.1083/jcb.201506	in press		Thailand
Hoang TA, Nguyen TN, Ueda S, Le QP, Tran TT, Nguyen TN, Dao TV, Tran MT, Le TT, Le TL, Nakayama T, Hirai I, Do TH, Vien QM, Yamamoto Y: Common findings of blaCTX-M-55-encoding 104–139 kbp plasmids harbored by extended-spectrum $\beta$ -lactamase-producing Escherichia coli in pork meat, wholesale market workers, and patients with urinary tract infection in Vietnam. Curr Microbiol. 74(2), 203–211, 2017.	doi: 10.1007/s00284-017-1395-7	published		Japan
Miyagi K, Sano K, Hirai I: Sanitary evaluation of domestic water supply facilities with storage tanks and detection of Aeromonas, enteric and related bacteria in domestic water facilities in Okinawa Prefecture of Japan. Water Res. 119, 171–177, 2017.	doi: 10.1016/j.watres.2017.04.002.	published		Japan

2 Total

## Lists of Achievements and Implemented Activities

### 2. presentations at Academic Conferences etc. (Seminars, Workshops, Symposia)

#### 【Notes】

Please fill in **only the achievements of this project** by country in order of presentation date.  
The information on this form is only disclosable. Please submit Non-disclosable information in a separate file.

#### 2. 1 Conference Presentations (Joint Presentations among Research Teams)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location, etc.
March 4, 2018	Guest/Invited Speaker	Taro Kagaku, "xxx", yyy, Tokyo,
March 27–29, 2018	Poster Session	Noriko Nakanishi, Ryohei Nomoto, Kanako Sato, Chihiro Koike, Mari Kusuki, Tatsuya Nakamura, Katsumi Shigemura, Toshiro Shirakawa, and Issei Tokimatsu and Kayo Osawa, "Analysis of antimicrobial resistance mechanism in successive infections of <i>Pseudomonas aeruginosa</i> .", The 91st Annual Meeting of Japanse Society for Bacteriology, Fukuoka, Japan. Fukuoka, Japan
March 27–29, 2018	Poster Session	Rosantia Sarassari, Usman Hadi, Itaru Hirai, Kuntaman Kuntaman, "The pattern of ESBL producing Gut flora among hospitalized patients and community in Surabaya.", The 91st Annual Meeting of Japanse Society for Bacteriology, Fukuoka, Japan. Fukuoka, Japan
Aug. 31–Sep. 4, 2016	Poster Session	Akiho Kanaida, Kayo Osawa, Katsumi Shigemura, Alpha Fardah, Dadik Raharjo, Eddy Bagus Wasito, Sugeng Harijono, Subijanto Marto Sudarmo, Toshiro Shirakawa, "Dissemination of extended-spectrum $\beta$ -lactamase producing <i>Escherichia coli</i> in Indonesia ", The 32nd world congress of biomedical labolatory science, Kobe, Japan

3 Total

## 2. 2 Conference Presentations (by Single Team)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location etc.	Country name of the team
March 4, 2018	Guest/Invited Speaker	Taro Kagaku, "xxx", yyy, Tokyo,	Thailand
May 31st–June 2nd, 2018	Oral Presentation	Kouta Hamamoto and Itaru Hirai, "Genetic characterization of chromosomally-located blaCTX-M of Escherichia coli producing CTX-M type extended spectrum beta-lactamase.", The 92nd Annual Meeting of the Japanese Association for Infectious Disease, Okayama, Japan	Japan
June 7–11, 2018	Poster Session	Y Yamamoto, R. Kawahara, Y Fujita, T Sasaki, I Hirai, DT Khong, HT Tran, TN Hoang, BX Nguyen, TN Nguyen, "Extremely high prevalence of colistin-resistant Escherichia coli with mcr in Healthy residents in Vietnam.", ASM microbe 2018 Atlanta, USA.	Japan
June 7–11, 2018	Poster Session	K Hamamoto, T Tokunaga, N Yagi I Hirai, "Frequent transposition of plasmid blaCTX-M to chromosome as a potential contributing factor for increasing prevalence of Escherichia coli possessing blaCTX-M.", ASM microbe 2018, Atlanta, USA.	Japan
March 27–29, 2018	Poster Session	Kazufumi Miyagi, Noriaki Shimoji, Itaru Tamaki, Ayumi Uechi, Itaru Hirai, "Elucidation of source of infection and character of clinical and environmental isolates of Aeromonas.", The 91st Annual Meeting of Japanese Society for Bacteriology, Fukuoka, Japan.	Japan
March 27–29, 2018	Poster Session	Kouta Hamamoto, Tshiro Tokunaga, Nobuyoshi Yagi, Itaru Hirai, "Transfer frequency of plasmid blaCTX-M to chromosome in Escherichia coli.", The 91st Annual Meeting of Japanese Society for Bacteriology, Fukuoka, Japan.	Japan
March 27–29, 2018	Poster Session	Seina Higa, Kouta Hamamoto, Yasuaki Yakabi, Rosantia Sarassari, Yasuko Koja, Itaru Hirai, "Characterization of ESBL-producing bacteria isolated from healthy individuals in Okinawa prefecture.", The 91st Annual Meeting of Japanese Society for Bacteriology, Fukuoka, Japan.	Japan
June 1–5, 2017	Poster Session	K Hamamoto, and I Hirai, "The role of chromosomal blaCTX-M in distribution of Escherichia coli producing CTX-M type extended-spectrum $\beta$ -lactamase isolated from a hospital of Okinawa, Japan.", ASM microbe 2017, New	Japan
March 19–21, 2017	Guest/Invited Speaker	Itaru Hirai, Kouta hamamoto, "Transfer of antimicrobial resistance (AMR) gene into bacterial genome by insertion sequence.", The 90th Annual Meeting of Japanese Society for Bacteriology, Sendai, Japan.	Japan
March 19–21, 2017	Oral Presentation	Kouta, Hamamoto, Itaru Hirai, "The role of chromosomal blaCTX-M in long-term detection of Escherichia coli clinical isolates producing ESBL.", The 90th Annual Meeting of Japanese Society for Bacteriology, Sendai, Japan.	Japan
March 23–25, 2016	Oral Presentation	Itaru Hirai, Yoshimasa Yamamoto, "Community-level distribution of extended-spectrum beta-lactamase-producing bacteria.", The 89th, Annual Meeting of Japanese Society for Bacteriology, Osaka, Japan	Japan
March 23–25, 2016	Oral Presentation	Kouta Hamamoto and Itaru Hirai, "High detection rate of chromosomal blaCTX-M in Escherichia coli isolates.", The 89th, Annual Meeting of Japanese Society for Bacteriology, Osaka, Japan	Japan

11 Total

## Lists of Achievements and Implemented Activities

### 3. Workshops, Seminars, Symposia and Other Events (Organized by the Project)

**【Notes】**  
Please fill in **only the achievements of this project** in order of event date.  
The information on this form is only disclosable. Please submit Non-disclosable information in a separate file.

Event duration	Name of Organizer	Title of the Event	Location (Country, City, Venue)	Number of Participants (Including Team Members)	Overview
Mar 4-16, 2018	Taro Yamada	○○○○	Germany, Hamburg, ○○○○	10	
16-Nov-16	Itaru Hirai, Kuntaman Kuntaman	Kick-off meeting	Indonesia, Surabaya, Airlangga University	10	The e-ASIA collaboration started, therefore, the kick-off meeting was held.

1

Total

## Lists of Achievements and Implemented Activities

### 4. Record of Research Exchanges

#### 【Notes】

Please fill in the record of **research exchange only of this project**.  
 "Duration of exchange" is not the number of days stayed on the site, but the number of days from departure to return home.  
 The information on this form is only disclosable. Please submit Non-disclosable information in a separate file.

Date of Departure	Date of Return	Last Name & First Name	Country of Affiliation	Affiliation	Position	Exchange Destination (Country, City, Research Organization etc.)	Description of Exchange Content/Purpose	Duration of Exchange (autocompleted)
January 6, 2016	January 16, 2016	Taro Yamada	Japan	Yamada University	Professor	NANOTEC, NECTEC, Bangkok	〇〇	11
August 9, 2016	August 11, 2016	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya	Research meeting	3
November 15, 2016	November 17, 2016	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya	Research meeting and Kick-off meeting	3
November 15, 2016	November 17, 2016	Toshiro Shirakawa	Japan	Kobe University	Professor	Indonesia, Surabaya	Research meeting, Kick-off meeting and	3
November 15, 2016	November 17, 2016	Kazufumi Miyagi	Japan	University of the Ryukyus	Research associate	Indonesia, Surabaya	Research meeting and Kick-off meeting	3
November 15, 2016	November 17, 2016	Kouta Hamamoto	Japan	University of the Ryukyus	Ph.D candidate	Indonesia, Surabaya	Research meeting and Kick-off meeting	3
January 8, 2017	January 10, 2017	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya	Research meeting	3
February 21, 2017	February 23, 2017	Toshiro Shirakawa	Japan	Kobe University	Professor	Indonesia, Surabaya	Research meeting and sampling	3
September 19, 2017	September 22, 2017	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya	Research meeting	4
November 21, 2017	November 23, 2017	Toshiro Shirakawa	Japan	Kobe University	Professor	Indonesia, Surabaya	Research meeting and sampling	3
January 14, 2018	January 16, 2018	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya	Research meeting	3
January 14, 2018	January 16, 2018	Rosantia Sarassari	Japan	University of the Ryukyus	Ph.D candidate	Indonesia, Surabaya	Research meeting	3
February 11, 2018	February 14, 2018	Rosantia Sarassari	Japan	University of the Ryukyus	Ph.D candidate	Indonesia, Surabaya	Research meeting	4
February 12, 2018	February 14, 2018	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya	Research meeting	3
February 18, 2018	February 22, 2018	Shohiro Kinoshita	Japan	Kobe University	Researcher	Indonesia, Surabaya, Airlangga University	Research meeting, experiments and sampling	5
								0
								0

Total (Person) 14

Total (Person-day) 46

Lists of Achievements and Implemented Activities

5. Patent Applications

[Notes]  
Please fill in **only the achievements of this project** by country in order of presentation date.  
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5. 1 Independent Applications by Single Team

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)	Country Name of the Team
WO20xx-xxxxxx		January 21, 2016	○○ Univ、Univ.of xx	WO/2016/xxxxxx	○○○○、○○・○○	PCT	WO20xx-xxxxxx (20xx.xx.xx)	Thailand

Total (Number of Application)

Total (Number of Registration)

5. 2 Joint Applications

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)
WO20xx-xxxxxx		January 21, 2016	○○ Univ、Univ.of xx	WO/2016/xxxxxx	○○○○、○○・○○	PCT	WO20xx-xxxxxx (20xx.xx.xx)

Total (Number of Application)

Total (Number of Registration)



# Lists of Achievements and Implemented Activities

## 6. Awards

**【Notes】**

Please fill in **only the achievements of this project** by country in order of date of Award.  
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Date of Award	Name of Award	Recipient	Remarks	Country Name of the Team
December 24, 2015	〇〇 Prize	Taro Yamada		Thailand

0

Total