

*e-ASIA Joint Research Program
Final 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 1, 2016 ~ March 1, 2020

3. Research Team :

■ **Japan team** (up to 6 people including the Principal Investigator)

Funding period: August, 1, 2016 - March, 31, 2020

Total Funded Amount (in Local Currency): 24,000,000

	Name	Position	Affiliation	Role in the project
PI	Itaru Hirai	Professor	University of the Ryukyus	Project management, and molecular analysis of multidrug resistance Gram-negative bacteria
Collaborator	Toshiro Shirakawa	Professor	Kobe University	Characterization of clinical isolates.
Collaborator	Kayo Osawa	Professor	Kobe Tokiwa University	Characterization of clinical isolates.
Collaborator	Kouta Hamamoto	Graduate student	University of the Ryukyus	Molecular analysis of drug resistance genes
Collaborator	Rosantia Sarassari	Graduate student	University of the Ryukyus	Characterization of multidrug resistance bacterial isolates
Collaborator	Nobuyoshi Yagi	Graduate student	University of the Ryukyus	Establishing analytical method for drug resistance genes inserted into bacterial chromosome
Total number of participants including students: 12				

■ **Indonesia team** (up to 6 people including the Principal Investigator)

Funding period: August, 1, 2016 – March, 31, 2020

■ Total Funded Amount (in Local Currency): 30,000,000

	Name	Position	Affiliation	Role in the project
PI	Kuntaman Kuntaman	Professor	Faculty of Medicine, Universitas Airlangga	Project management, field project development and

				sample collection
Collaborator	Siti Rochmanah Oktaviani Sulikah MD	Graduate student	Universitas Airlangga	Sample collection in the field
Collaborator	Linda Dewanti, MD., PhD	Lecturer	Universitas Airlangga	Sample collection in the field
Collaborator	Sulistiyawati, MD., PhD	Lecturer	Universitas Airlangga	Sample collection in the field
Total number of participants including students: 4				

■ **Vietnam team** (up to 6 people including the Principal Investigator)

Funding period: Month, Day, Year - Month, Day, Year

Total Funded Amount (in Local Currency): None

	Name	Position	Affiliation	Role in the project
PI	Bui Thi Mai Huong	Dept. Head and Senior researcher	Dept. of Food Microbiology and Molecular Biology, National Institute of Nutrition	Project management, sample collection, data analysis
Collaborator	Ha Thi Tuong Van	Researcher	Dept. of Food Microbiology and Molecular Biology, National Institute of Nutrition	Sample collection and primary characterization of multidrug resistance Gram-negative bacteria
Collaborator	Kieu Minh Duc	Researcher	Dept. of Food Microbiology and Molecular Biology, National Institute of Nutrition	Sample collection and epidemiological analysis of multidrug resistance Gram-negative bacteria
Collaborator	Nguyen Anh Tuyet	Researcher	Dept. of Food Microbiology and Molecular Biology, National Institute of Nutrition	Sample collection and primary characterization of multidrug resistance Gram-negative bacteria
Total number of participants including students: 4				

4. Summary of the joint research

Antimicrobial resistant (AMR) bacteria, such as Enterobacteriaceae producing extended-spectrum β -lactamase (ESBL) have been spread. Regarding *bla*_{CTX-M}, a gene encoding CTX-M type ESBL, insertion sequence (IS) such as *ISEcp1* is observed at the upstream of *bla*_{CTX-M}. *ISEcp1* can transfer its downstream *bla*_{CTX-M} among antimicrobial resistance plasmids and from an antimicrobial resistance plasmid to bacterial chromosome. Generally, AMR genes on chromosome could be more stable. Therefore, identification of AMR gene locations is important for control spread of AMR bacteria. In this research collaboration, we aimed to establish a cost effective and high throughput analytical method for determination of AMR gene location, and to apply the established method for analysis of CTX-M type ESBL-producing bacterial isolates which obtained from Japan, Vietnam and Indonesia.

This research collaboration collected about 170 bacterial strains from Japan, 500 or more strains from Indonesia and Vietnam, respectively, and total of more than 1200 bacterial strains were analyzed. In addition, about 60 colistin-resistant strains (plasmid colistin-resistant gene *mcr-1* positive strain, etc.) were used and the applicability to drug resistance genes other than *bla*_{CTX-M} was examined. At present, chromosomally transferred *bla*_{CTX-M} was detected in 22.7% of the analyzed 666 bacterial isolates.

5. Outputs and Anticipated Outcomes of Joint Research

5-1 Scientific achievements and implemented activities of the joint research

i) Establishment of a cost effective and high throughput analytical method for determination of AMR gene location.

We adopted homemade (HM) adaptors containing “barcode sequence” and adaptor ligation mediated PCR (ALM-PCR) for amplification of DNA fragments containing upstream region adjacent of *ISEcp1-bla*_{CTX-M}. The amplified DNA fragments were analyzed by the Nanopore sequencer (Fig. 1). At this point, the protocol allowed us to determine at least 96 locations of *bla*_{CTX-M} in one run of Nanopore sequencer. Evaluation by using colistin resistant bacteria possessing *mcr-1* suggested the established protocol was applicable to determination of other AMR gene locations.

ii) Detection and characterization of drug-resistant bacteria carrying chromosomally located AMR gene.

By using the established AMR gene location determination method, chromosomally located *bla*_{CTX-M} were

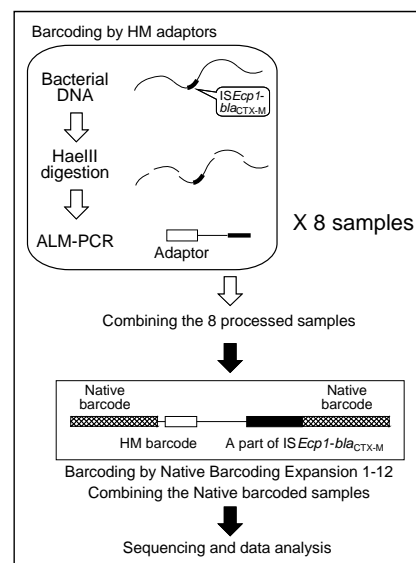


Fig. 1 Detection flow of AMR gene location by ALM-PCR and Nanopore sequencing.

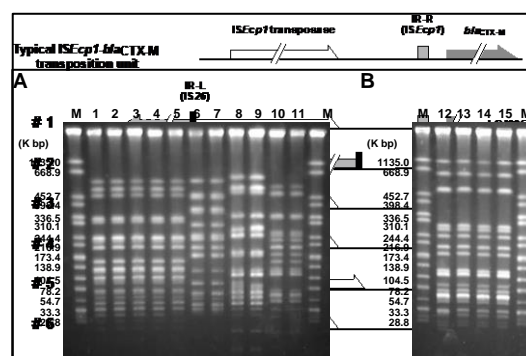


Fig. 3 Pulsed field gel electrophoresis of *E. coli* isolates possessing *bla*_{CTX-M} at same chromosomal region.

detected in Enterobacteriaceae isolates, mainly *Escherichia coli* isolates, which were obtained from healthy asymptomatic Vietnamese, hospitals in Indonesia and Japan, and Indonesian medical student. The detection rates were varied among the sample population (approximately 10 to 60% in this project). It was observed that *ISEcp1* coding sequence in many plasmids were impaired by insertion(s) by other gene(s) such as IS26 (Fig. 2). However, genetic structure was intact in the detected chromosomally transferred *ISEcp1-bla_{CTX-M}*. Analysis using an *E. coli* model strain indicated that exactly same inverted repeat L (IR-L) sequence and intact *ISEcp1-bla_{CTX-M}* was used for the transposition from plasmid to chromosome.

iii) Role(s) of chromosomally located AMR gene in spread of AMR bacteria.

Genetically similar CTX-M type ESBL-producing isolates possessing chromosomally located *bla_{CTX-M}* at exactly same region were detected in CTX-M type ESBL-producing *E. coli* isolates which were obtained from several different individuals or patients of Japan, Indonesia and Vietnam (Fig. 3). In addition, we observed several *E. coli* clinical isolates possessing chromosomally transferred *bla_{CTX-M}* at exactly same chromosomal region had been detected in a Japanese hospital for at least 11 months. It was suggested that chromosomally transferred *bla_{CTX-M}* in *E. coli* experimental model was more stable than plasmid *bla_{CTX-M}* when there was no selection pressure by antibiotics, suggesting stability of chromosomally located *bla_{CTX-M}* in bacterial cells. Stability of AMR gene might be a contributing factor for spreading AMR bacteria, even though responsible mechanism had not been well explained.

Furthermore, we observed that a potential recurrent transposition of *ISEcp1-bla_{CTX-M}* from a chromosomal region to another chromosomal region in clinical *E. coli* isolates (Fig. 4).

Taken together, these observations implied that chromosomally transferred *bla_{CTX-M}* was stably maintained in bacterial isolates, and that *ISEcp1-bla_{CTX-M}* as a reservoir of transferrable *bla_{CTX-M}* could be source of transfer of *bla_{CTX-M}* into other genetic regions.

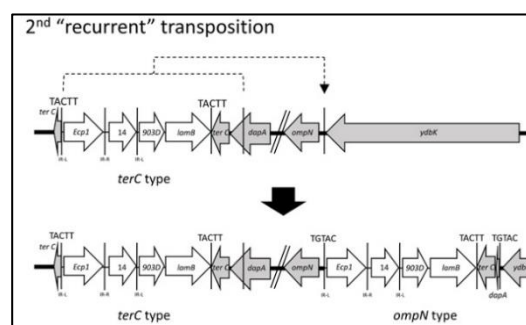


Fig.4 Recurrent transposition of *ISEcp1-bla_{CTX-M}* in a clinical isolate.

5-2 Synergistic effects of the international joint research

Healthcare policy and healthcare system is different in each country; consequently, situation regarding AMR issue is unique to each country. AMR bacteria has been internationally distributed. Thus, exchange of information and research materials regarding AMR issue would be essential. In this regard, international collaboration such as the e-ASIA JRP had been facilitated communication among the three countries and transporting research materials such as AMR bacterial isolates.

In this research collaboration, we utilized cost effective technologies, such as the nanopore sequencer and homemade adapters, that can be introduced

in any country. These technologies are likely to be adopted in Indonesia and Vietnam in the future and can contribute to improving the detection and characterization of AMR bacteria especially at healthcare facilities.

During research period of this research collaboration project, two Indonesian Ph.D. candidates were invited to University of the Ryukyus as the Japanese government (MEXT) scholars. These young researchers are learning and acquiring advanced molecular microbiological skills in Japan and expected to become leaders in clinical microbiology as MD, Ph.D. after coming back to Indonesia. Such nurturing of researchers is fundamental for potential international joint research in the future and is expected to bring further synergistic effects in this research field.

When visiting Indonesian and Vietnamese research institutes, Japanese researchers provided lectures that could contribute to improving the research capabilities of Indonesian and Vietnamese young researchers.

5-3 Broader impacts including contribution to society

Regarding AMR bacteria, WHO and many countries have action plans to control the distributed AMR bacteria. For example, proper use of antibiotics by avoiding abuse and heavy use is set as one of the major points in the action plans to decrease the detection numbers of drug-resistant bacteria. However, proper use of antibiotics may not reduce detection rates of bacterial strains carrying the chromosomal AMR gene(s), because chromosomal AMR genes would be considered stable regardless of antibiotics. The established method in this research collaboration for detecting chromosomal ARM gene(s) can be applicable to other AMR genes. Its detection cost is not high, rather, reasonable. Introduction of the AMR gene detection method of this project into the Southeast Asian countries would contribute to surveillance of bacteria carrying chromosomal AMR gene(s) in this region which AMR bacteria have been well distributed. In addition to the surveillance, strengthening of awareness about AMR is also one of the above action plans. Generally, it is not easy to notice the presence of AMR bacteria. The surveillance of AMR bacteria by using the established AMR gene detection method would contribute to providing information regarding AMR bacteria for strengthening awareness about AMR bacteria.

5-4 Development and sustainability of the cooperation

As an approach after this research collaboration, the current MOU (Memorandum of Understanding) between faculties of Medicine would be planned to be expanded to a MOU between two universities, namely Airlangga University and University of the Ryukyus, for researcher exchange. In addition, communication between the two universities have been started for student exchanging programs, such as a sandwich program for Ph.D candidates and short-term visit program for Indonesian students.

Based on the achievement of this research collaboration, a research proposal for the Science and Technology Research Partnership for Sustainable Development (SATREPS) program is preparing by Japanese and Vietnamese researchers. For this research proposal, discussion between

researchers of the two countries and Vietnamese Ministry of Health has been started.

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

The method for detecting chromosomal drug resistance genes which was established in the research collaboration was based on the environmental metabarcoding. Therefore, the established detection method can be applicable to characterization of other various drug resistance genes. Which leads to the establishment of comprehensive detection methods of drug resistance genes in various samples, and it becomes possible to detect the presence of a drug resistance genes in various places such as medical facilities, environment, and food. In addition, by conducting environmental metabarcoding analysis at the same time, it will be possible to catalog the antibiotic resistance genes and bacterial microflora in the sample. These research activities will provide important information for promoting cost-effective intervention program, such as strengthening awareness to control distributed AMR bacteria in the communities. Based on the achievement in this research collaboration, several research proposals are preparing. It includes the SATREPS and the bilateral program by the Japan Society for the Promotion of Science. If these research proposals will be accepted, it will provide sufficient opportunity for researchers, especially for younger researchers to promote research collaboration, researcher exchange and the Japanese government (MEXT) scholar ship.

7. Scientific Achievements and Implemented Activities (Publication, Research Exchange, Workshop, etc.)

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8. Recommendations and Comments to the Program

During the research period of this research collaboration, there was no major problem which was difficult to solve. Therefore, there is not specific major recommendation and comments to this program. Even though, we believe the subsequent second phase research by the same research group might enable young researchers to have opportunities for further research and researcher exchange from other countries.

9. Others (agenda of workshop, photos of research teams, meetings, and etc.)

This research collaboration had three meetings in the research period. The agendas of the meetings are follows.

1. Kick off meeting

**Kick-off Meeting
Research Collaboration**
Faculty of Medicine, Universitas Airlangga, Indonesia
School of Health Sci, Faculty of Medicine, University of the Ryukyus, Japan
Dept. of Food Microbiol. and Mol. Biol, National Institute of Nutrition, Vietnam
Faculty of Medicine, Kobe University, Japan

Research Funded by: e-ASIA Joint Research Program (e-ASIA JRP)

Wednesday, Nov 16, 2016

Time	Agenda	Note
09.00	Opening remarks Dean, Faculty of Medicine Universitas Airlangga	
09.30	Introduction of attendees (All attendees)	Chair/Moderator Prof. Itaru Hirai
09.45	Outline of Collaborative e-ASIA project <i>Prof. Itaru Hirai</i>	
	Research from each countries	Moderator Prof. Itaru Hirai
10.15	Research from Okinawa, Japan (Mr. Kouta Hamamoto)	
10.30	Research from Kobe, Japan (Prof. Shirakawa)	
10.45	Research from Vietnam (Prof. Itaru Hirai)	
11.00	Research from Indonesia (Prof. Kuntaman)	
11.15	Discussion	
12.00	Lunch	

2. Midterm meeting

The agenda of the e-ASIA meeting.

Date and time: 9:00 am on Jan 9th, 2019

Venue: National Institute of Nutrition, Vietnam

1. Opening remarks
2. Research progress from each group
 - 2-1. Research from Japan
 - 2-1-1. Chromosomally-located *bla*_{CTX-M} and its analytical methods using next generation sequencer. (Hirai)
 - 2-1-2. Characterization of chromosomally-located *bla*_{CTX-M} in *Escherichia coli* isolates producing ESBL. (Mr. Hamamoto)
 - 2-2. Research from Indonesia
 - 2-2-1. The roadmap of the study of ESBL producing bacteria in Surabaya. (Prof. Kuntaman)
 - 2-2-2. Prevalence of ESBL-Producing *E. coli* strains harboring chromosomal *bla*_{CTX-M-15} in Surabaya, Indonesia. (Prof. Shirakawa)
 - 2-2-3. Characterization of Extended Spectrum β Lactamase (ESBL)-producing Enterobacteriaceae in Indonesia. (Dr. Santi)
 - 2-3. Research from Vietnam
 - 2-3-1. Prevalence and Antimicrobial Resistance of Enterococci Isolated from healthy Vietnamese individual. (Mr. Duc)
 - 2-3-2. Characterization of Extended Spectrum β -Lactamase (ESBL)-producing Enterobacteriaceae in Vietnamese community. (Mr. Yakabi)
3. Discussion (if it will be needed)
4. Closing remarks

3. Closing meeting

The agenda of the e-ASIA meeting.

Date and time: 9:00 am on Feb 27th, 2020

Venue: University of the Ryukyus, Okinawa, Japan

1. Opening remarks

2. Research achievement from each group.

Japan side

2-2. Mr. Hamamoto: Frequent transposition of *bla*_{CTX} from plasmid to chromosome by *ISEcp1*-mediated transposition as a potential contributing factor for increasing prevalence of CTX-M type ESBL-producing bacteria.

2-3. Mr. Yagi: Establishment of high through-put detection method of *bla*_{CTX-M} location on the bacterial DNA, and *bla*_{CTX-M} characterization based on the upstream construction of *bla*_{CTX-M}.

Indonesia side

2-4. Prof. Kuntaman

2-5. Prof. Shirakawa: Molecular characteristics of ESBL-producing *E. coli* isolated from patients with urinary tract infection in Surabaya, Indonesia.

2-6. Dr. Santi: Characterization of ESBL-producing Enterobacteriaceae isolated from Patients, Families and medical students in Surabaya, Indonesia.

2-7. Dr. Fikri: Progress of Molecular Characterization of ESBL-producing Enterobacteriaceae Isolated from Community Setting in Surabaya, Indonesia.

Vietnam side

2-8. Dr. Huong: Prevalence, mechanisms, and zoonotic aspects of antimicrobial resistant *E. faecalis* and *E. faecium* in Vietnamese pigs, pig farmers and community individuals.

3. Discussion (if it will be needed)

4. Summary

Lists of Achievements and Implemented Activities

1. Original Publication of Articles etc.

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.)
Bagus Wasito E, Shigemura K, Osawa K, Fardah A, Kanaida A, Raharjo D, Kuntaman K, Hadi U, Harjono 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.jjgar.2017.09.014.	published	
Kuntaman K, Shigemura K, Osawa K, Kitagawa K, Sato K, Yamada N, Nishimoto K, Yamamichi F, Rahardjo D, Hadi U, Mertaniasih NM, Kinoshita S, Fujisawa M, Shirakawa T, Occurrence and characterization of carbapenem-resistant Gram-negative bacilli: A collaborative study of antibiotic-resistant bacteria between Indonesia and Japan., Int J Urol., 2018, 25(11), 966–972.	doi:10.1111/iju.13787.	published	
Rosantia S, Higa T, Yagi N, Tokunaga T, Higa S, Yakabi Y, Shirakawa T, Kuntaman K, Hirai I, Characterization of CTX-M-type-extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae isolated from Indonesian undergraduate medical students of a university in Surabaya, Indonesia., J Infect Chemother., 2020 Mar 6., pii: S1341–321X(20)30024–6.	doi: 10.1016/j.jjiac.2020.01.010.	published	

4 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
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 β -lactamase-producing <i>Escherichia coli</i> in pork meat, wholesale market workers, and patients with urinary tract infection in Vietnam., <i>Curr Microbiol.</i> , 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 <i>Aeromonas</i> , enteric and related bacteria in domestic water facilities in Okinawa Prefecture of Japan., <i>Water Res.</i> , 119, 171-177, 2017.	doi: 10.1016/j.watres.2017.04.002.	published		Japan
Naelasari DN, Koendhori EB, Dewanti L, Sarassari R, Kuntaman K, The prevalence of extended spectrum beta-lactamase (ESBL) producing gut bacterial flora among patients in Dr. Soetomo hospital and primary health center in Surabaya., <i>Folia Medica Indonesiana</i> , 2018, 54(4), 256-260.	doi: 10.20473/fmi.v54i4.10708	published		Indonesia
Yamamoto Y, Kawahara R, Fujiya Y, Sasaki T, Hirai I, Khong DT, Nguyen TN, Nguyen BX., Wide dissemination of colistin-resistant <i>Escherichia coli</i> with the mobile resistance gene <i>mcr</i> in healthy residents in Vietnam., <i>J Antimicrob Chemother.</i> , 2019, 74(2), 523-524.	doi: 10.1093/jac/dky435.	published		Japan
Miyagi K, Hirai I, A survey of extended-spectrum β -lactamase-producing Enterobacteriaceae in environmental water in Okinawa Prefecture of Japan and relationship with indicator organisms., <i>Environ Sci Pollut Res Int.</i> , 2019, 26(8), 7697-7710.	doi: 10.1007/s11356-019-04189-z.	published		Japan
Higa S, Sarassari R, Hamamoto K, Yakabi Y, Higa K, Koja Y, Hirai I, Characterization of CTX-M type ESBL-producing Enterobacteriaceae isolated from asymptomatic healthy individuals who live in a community of the Okinawa prefecture, Japan., <i>J Infect Chemother.</i> , 2019, 25(4), 314-318.	doi: 10.1016/j.jjiac.2018.09.005.	published		Japan
Hamamoto K, Hirai I, Characterisation of chromosomally-located blaCTX-M and its surrounding sequence in CTX-M-type extended-spectrum β -lactamase-producing <i>Escherichia coli</i> isolates., <i>J Glob Antimicrob Resist.</i> , 2019, 17, 53-57.	doi: 10.1016/j.jjgar.2018.11.006.	published		Japan
Yamaguchi T, Kawahara R, Hamamoto K, Hirai I, Khong DT, Nguyen TN, Tran HT, Motooka D, Nakamura S, Yamamoto Y, High Prevalence of Colistin-Resistant <i>Escherichia coli</i> with Chromosomally Carried <i>mcr</i> -1 in Healthy Residents in Vietnam., <i>mSphere</i> . 2020, 5(2), pii: e00117-20.	doi: 10.1128/mSphere.00117-20.	published		Japan
Hamamoto K, Tokunaga T, Yagi N, Hirai I, Characterization of blaCTX-M-14 transposition from plasmid to chromosome in <i>Escherichia coli</i> experimental strain., <i>Int J Med Microbiol.</i> , 2020, 310(2), 151395.	doi: 10.1016/j.ijmm.2020.151395.	published		Japan

9 Total

Lists of Achievements and Implemented Activities

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

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

Date	Type of Presentation	Speaker, "Title", Conference Name, Location, etc.
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 β -lactamase producing <i>Escherichia coli</i> in Indonesia", The 32nd world congress of biomedical laboratory science, Kobe, Japan.
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 Japanese Society for Bacteriology, 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 Japanese Society for Bacteriology, Fukuoka, Japan.
April 23–25, 2019	Poster Session	Rosantia Sarassari, Takuya Higa, Kuntaman Kuntaman, Itaru Hirai, "Characterization of ESBL-producing <i>Escherichia coli</i> in medical students", The 92nd Annual Meeting of Japanese Society for Bacteriology, Sapporo, Japan.
June 20–24, 2019	Poster Session	Bui Thi Kim Ngan, Shuhei Ueda, Nobuyoshi Yagi, Kouta Hamamoto, Le Danh Tuyen, Bui Thi Mai Huong, Itaru Hirai, "Clonal Diversity of blaCTX-M-Positive <i>Escherichia coli</i> Isolates Carried in Healthy Vietnamese Individuals.", ASM Microbe 2019, San Francisco, CA, USA.
Feb 19–21, 2020	Poster Session	Fikri Widyatama, Rosantia Sarassari, Takuya Higa, Kouta Hamamoto, Kuntaman Kuntaman, Itaru Hirai, "Characterization of ESBL-producing <i>Escherichia coli</i> in Surabaya, Indonesia.", The 93rd Annual Meeting of Japanese Society for Bacteriology, Nagoya, Japan.
Feb 19–21, 2020	Poster Session	Rosantia Sarassari, Takuya Higa, Nobuyoshi Yagi, Kuntaman Kuntaman, Itaru Hirai, "Characterization of CTX-M type ESBL-producing <i>E. coli</i> from ICU and non-ICU ward in Indonesia.", The 93rd Annual Meeting of Japanese Society for Bacteriology, Nagoya, Japan.

2. 2 Conference Presentations (by Single Team)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location etc.	Country name of the team
March 23–25, 2016	Poster Session	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
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 19–21, 2017	Poster Session	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 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
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 β -lactamase isolated from a hospital of Okinawa, Japan.", ASM microbe 2017, New Orleans, USA	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
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	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
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
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
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
April 23–25, 2019	Poster Session	Kouta Hamamoto, Toshiro Tokunaga, Nobuyoshi Yagi, Itaru Hirai, "Observation of transfer frequency of ISEcp1–blaCTX-M–14 transposition unit on plasmid to chromosome using Escherichia coli model strain.", The 92nd Annual Meeting of Japanese Society for Bacteriology, Sapporo, Japan.	Japan
April 23–25, 2019	Poster Session	Toshiro Tokunaga, Rosantia Sarassari, Yasuaki Yakabi, Itaru Hirai, "Stability of chromosomally-located blaCTX-M in absence of antibiotic selective pressure.", The 92nd Annual Meeting of Japanese Society for Bacteriology, Sapporo, Japan.	Japan
April 23–25, 2019	Poster Session	Takuya Higa, Rosantia Sarassari, Kuntaman Kuntaman, Itaru Hirai, "Comparison of extended-spectrum beta lactamase producing Escherichia coli in healthcare-associated facilities.", The 92nd Annual Meeting of Japanese Society for Bacteriology, Sapporo, Japan.	Japan

April 23–25, 2019	Poster Session	Nobuyoshi Yagi, Kouta Hamamoto, Itaru Hirai, “Establish the analysis method of the blaCTX–M location by Nanopore sequencing.”, The 92nd Annual Meeting of Japanese Society for Bacteriology, Sapporo, Japan.	Japan
April 23–25, 2019	Poster Session	Kazufumi Miyagi, Shogo Shimoji, Rina Tahara, Noriaki Shimoji, Itaru Tamaki, Ayumi Uechi, Haruna Ohshiro, Asami Komiyama, Itaru Hirai, “Discrimination of pathogenicity and clones of clinical and environmental Aeromonas species.”, The 92nd Annual Meeting of Japanese Society for Bacteriology, Sapporo, Japan.	Japan
June 20–24, 2019	Poster Session	Kouta Hamamoto, Toshiro Tokunaga, Nobuyoshi Yagi, Itaru Hirai, “Molecular Feature in ISEcp1 Transposase as Potential Contributing Factor for High Transfer Frequency of blaCTX–M–14 Transposition Unit from Plasmid to Chromosome.”, ASM Microbe 2019, San Francisco, CA, USA.	Japan
Feb 19–21, 2020	Poster Session	Kouta Hamamoto, Toshiro Tokunaga, Nobuyoshi Yagi, Itaru Hirai, “ISEcp1–mediated transposition of AMR genes by usage of various right terminal sequence.”, The 93rd Annual Meeting of Japanese Society for Bacteriology, Nagoya, Japan.	Japan
Feb 19–21, 2020	Poster Session	Nobuyoshi Yagi, Itaru Hirai, “blaCTX–M horizontal transfer intra– and inter– families.”, The 93rd Annual Meeting of Japanese Society for Bacteriology, Nagoya, Japan.	Japan

Lists of Achievements and Implemented Activities

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

Event duration	Name of Organizer	Title of the Event	Location (Country, City, Venue)	Number of Participants (Including Team Members)	Overview
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.
9-Jan-19	Bui Thi Mai Huong, Itaru Hirai	Midterm meeting	Vietnam, Hanoi, National Institute of Nutrition	8	To discuss scientific progress in each country team, the midterm meeting was held.
27-Feb-20	Itaru Hirai	Closing meeting	Japan, Okinawa, University of the Ryukyus	7	The e-ASIA collaboration has ended in this Japanese budgetary year, 2019. Therefore, the closing meeting was held.

3 Total

Lists of Achievements and Implemented Activities

4. Record of Research Exchanges

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)
August 9, 2016	August 11, 2016	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya, Airlangga University	Research meeting	3
November 15, 2016	November 17, 2016	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya, Airlangga University	Research meeting and Kick-off meeting	3
November 15, 2016	November 17, 2016	Toshiro Shirakawa	Japan	Kobe University	Professor	Indonesia, Surabaya, Airlangga University	Research meeting, Kick-off meeting and sampling	3
November 15, 2016	November 17, 2016	Kazufumi Miyagi	Japan	University of the Ryukyus	Research associate	Indonesia, Surabaya, Airlangga University	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, Airlangga University	Research meeting and Kick-off meeting	3
January 8, 2017	January 10, 2017	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya, Airlangga University	Research meeting	3
February 21, 2017	February 23, 2017	Toshiro Shirakawa	Japan	Kobe University	Professor	Indonesia, Surabaya, Airlangga University	Research meeting and sampling	3
September 19, 2017	September 22, 2017	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya, Airlangga University	Research meeting	4
November 21, 2017	November 23, 2017	Toshiro Shirakawa	Japan	Kobe University	Professor	Indonesia, Surabaya, Airlangga University	Research meeting and sampling	3
January 14, 2018	January 16, 2018	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya, Airlangga University	Research meeting	3
January 14, 2018	January 16, 2018	Rosantia Sarassari	Japan	University of the Ryukyus	Ph.D candidate	Indonesia, Surabaya, Airlangga University	Research meeting	3
February 11, 2018	February 14, 2018	Rosantia Sarassari	Japan	University of the Ryukyus	Ph.D candidate	Indonesia, Surabaya, Airlangga University	Research meeting	4
February 12, 2018	February 14, 2018	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya, Airlangga University	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
August 4, 2018	August 8, 2018	Itaru Hirai	Japan	University of the Ryukyus	Professor	Vietnam, Hanoi, National Institute of Nutrition	Research meeting and sampling	5
August 4, 2018	August 8, 2018	Nobuyoshi Yagi	Japan	University of the Ryukyus	Graduate student	Vietnam, Hanoi, National Institute of Nutrition	Research meeting and sampling	5
August 4, 2018	August 8, 2018	Toshiro Tokunaga	Japan	University of the Ryukyus	Graduate student	Vietnam, Hanoi, National Institute of Nutrition	Research meeting and sampling	5
August 28, 2018	September 8, 2018	Sarassari Rosantia	Japan	University of the Ryukyus	Graduate student	Indonesia, Surabaya, Airlangga University	Research meeting and sampling	12
September 2, 2018	September 8, 2018	Itaru Hirai	Japan	University of the Ryukyus	Professor	Indonesia, Surabaya, Airlangga University	Research meeting and sampling	7
September 2, 2018	September 8, 2018	Takuya Higa	Japan	University of the Ryukyus	Graduate student	Indonesia, Surabaya, Airlangga University	Research meeting and sampling	7
January 8, 2019	January 10, 2019	Itaru Hirai	Japan	University of the Ryukyus	Professor	Vietnam, Hanoi, National Institute of Nutrition	Research meeting and Midterm meeting	3
January 8, 2019	January 10, 2019	Toshiro Shirakawa	Japan	Kobe University	Professor	Vietnam, Hanoi, Vietnam National Nutrition Institute	Research meeting and Midterm meeting	3
January 8, 2019	January 10, 2019	Kouta Hamamoto	Japan	University of the Ryukyus	Graduate student	Vietnam, Hanoi, National Institute of Nutrition	Research meeting and Midterm meeting	3
January 8, 2019	January 10, 2019	Sarassari Rosantia	Japan	University of the Ryukyus	Graduate student	Vietnam, Hanoi, National Institute of Nutrition	Research meeting and Midterm meeting	3
January 8, 2019	January 10, 2019	Yasuaki Yakabi	Japan	University of the Ryukyus	Graduate student	Vietnam, Hanoi, National Institute of Nutrition	Research meeting and Midterm meeting	3
January 8, 2019	January 10, 2019	Kuntaman Kuntaman	Indonesia	Airlangga University	Professor	University of the Ryukyus	Research meeting and Midterm meeting	3
May 15, 2019	May 18, 2019	Itaru Hirai	Japan	University of the Ryukyus	Professor	Vietnam, Hanoi, National Institute of Nutrition	Research meeting	4
July 17, 2019	July 20, 2019	Itaru Hirai	Japan	University of the Ryukyus	Professor	Vietnam, Hanoi, National Institute of Nutrition	Research meeting	4
December 5, 2019	December 7, 2019	Itaru Hirai	Japan	University of the Ryukyus	Professor	Vietnam, Hanoi, National Institute of Nutrition	Research meeting	3
February 23, 2020	February 28, 2020	Kuntaman Kuntaman	Indonesia	Airlangga University	Professor	University of the Ryukyus	Research meeting and Closing meeting	6

Total (Person)

30

Total (Person-day)

122

Lists of Achievements and Implemented Activities

5. Patent Applications

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

0

Total (Number of Application)

0

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)

0

Total (Number of Application)

0

Total (Number of Registration)

Lists of Achievements and Implemented Activities

6. Awards

Date of Award	Name of Award	Recipient	Remarks	Country Name of the Team

0

Total