This report should be submitted within 2 weeks after you return to Japan. Please do not change the formatting

（Abroad・Domestic）Internship report form（Student） 2020/04/17 (Year/Month/Day)

Name	Ruttana Pachanon
Laboratory	Division of Bioresources, Research Center for Zoonosis Control
Year (Grade)	D4
Internship institution	Laboratory of Food Microbiology and Food Safety, Department of Health and Environmental Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Japan
Internship period	Internship period: 03/16/2020 - 03/27/2020 (Departure Date from Sapporo:, Arrival Date in Sapporo:)
Purpose	・To gain experience by observing the workflow and teaching system of the laboratory of Food Microbiology and Food Safety. ・To learn about new techniques for antimicrobial resistance test of bacterial infections ・To build a good collaboration network with researchers in Japan.

- The reason why you chose this institute

Associate Professor Dr. Masaru Usui is working on antimicrobial resistance and teaching students for food microbiology and food safety. I was interested in bacteriology fields and his research work. Therefore, this institute may provide a good experience in the teaching and research, novel knowledge and new technique of food safety laboratory.

- Result of the activity (about 800 words, provide photos, tables and figures that clearly show the activities during the period)

1. Observed the workflow and joined the laboratory work at Laboratory of Food Microbiology and Food Safety

I met Dr. Usui and observed the workflow at the Laboratory of Food Microbiology and Food Safety. I also supported another researcher (Risa Tsunoda, who is D4 student as same as my laboratory at Division of Bioresources) for the isolation of bacteria from water samples at -80°C stock into Luria-Bertani (LB) agar plate and broth. Nevertheless, I discussed with Dr. Usui for bacteria isolation and identification such as methicillin resistance *Staphylococcus aureus* (MRSA) isolated from four swab samples of staffs in the Veterinary Medicine hospital at Rakuno Gakuen University as below.

DNAs were extracted for four MRSA samples as shown in Figure 1. Two genes (*femA* and *mecA*) were identified by PCR method as shown in Figure 2. The *femA* was used for confirming *Staphylococcus aureus*. And the *mecA* was used for confirming methicillin resistance. PCR reaction was performed in a total volume of 20 µl. Briefly, the PCR mixture (Quick taq polymerase, 0.1 µM of each primer, and 1 µl DNA template) was prepared. The PCR running cycles were consisted of an initial denaturation at 94°C for 2 min, and followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 68°C for 30 sec. The PCR products were analyzed by gel electrophoresis at 200 V for 30 min

and stain with ethidium bromide solution for 20 min. Then, the DNA bands were determined by UV light gel documentation.

Figure 1. DNA extraction by boiled method.

Resuspend the positive samples in 200 µl of TE buffer

↓

Boil at 100°C, 15 min

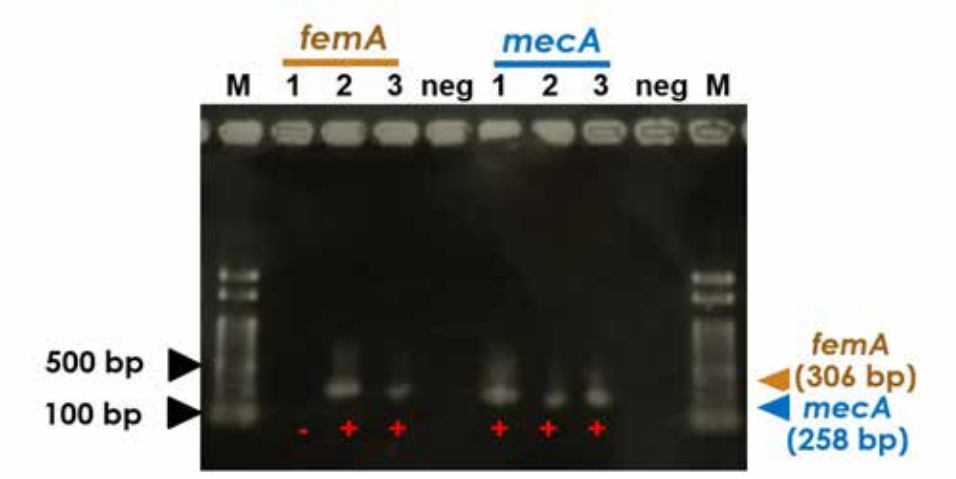
↓ Centrifuge at 15,000 x g, 10 min

Transfer the supernatant containing DNA ~150 µl into a new tube

↓

Keep genomic DNA solution at -20°C

Figure 2. PCR for *femA* and *mecA*.



2. Observed the teaching way of Dr. Usui and attended student's meeting:

I attended an undergraduate student's meeting with Dr. Usui in order to see the teaching way in the laboratory. And I also attended the lecture given by Dr. Usui on the topics as below.

- Antimicrobial resistance in bacterial infections situation in Japan
- Planktonic or biofilm
- Learn how to analyze metagenomic data of undergraduate student by using galaxy assembly and ResFinder which are available on the website

I learned how to discuss with undergraduate student and attended discussion such as management using antimicrobials in livestock and environment especially in milk from a daily cow in farm and compost manure.

3. Learning new techniques:

I learned new techniques for screening antimicrobial resistance of bacteria such as performing tolerance disk for determining the high persistence isolates of *Escherichia coli* with Dr. Usui (Figure 3). Moreover, Dr. Usui explained other techniques (Figure 4) such as colony count of *E. coli* on agar plate and using glass bead for spreading of bacteria.

For tolerance disk test as shown in Figure 3, three of high persistent strains, three tolerance/mutant strains, *E. coli* MG1655 as positive control and *E. coli* SRE94 as negative control were performed. Briefly, paper disks were cut to 6 mm by machine cutting containing a different concentration of antimicrobial agents; ofloxacin 10/100 µg/ml, amikacin 1000/10000 µg/ml and ampicillin 1000/10000 µg/ml. Then, 5 µl each of ofloxacin, amikacin and ampicillin were spotted on disk. The colony samples were suspended into 3 ml of NaCl 0.9% and adjusted to 0.5Mcfarland. The bacterial suspensions were spread by swab for rotating 3 times on LB agar and putted antimicrobial agents containing disk (ofloxacin 10/100 µg/ml, amikacin 1000/10000 µg/ml and ampicillin 1000/10000 µg/ml) and incubated at 37ºC for 18h. The antimicrobial agents of the incubated plates were removed and changed to 5 µl of 40% glucose disk and incubated at 37ºC for overnight.

4. Other activities:

I introduced the current research work and discussed my future research. For the last day, I summarized and presented internship report activities in this laboratory (Figure 5).

Figure 3. Tolerance disk for determining the high persistence isolates of *Escherichia coli*.

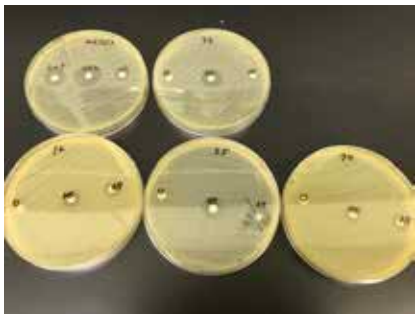
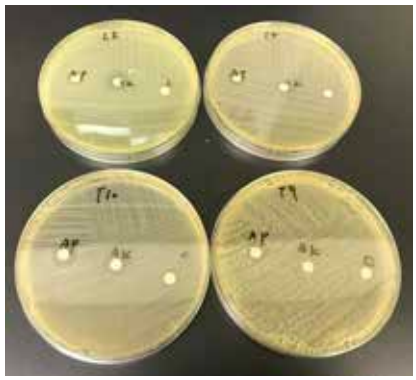
Antimicrobial agents	Strains	Results
- Ofloxacin 10 µg/ml - Amikacin 1000 µg/ml - Ampicillin 1000 µg/ml	- MG 1655 (positive; *E. coli*) - SRE94 (negative control) - SRE 12, 25, 78 (High persistent) - LF82_GPF, TRI 8, 9, 10 (mutant)	  - These results need more to see inhibition zone of the antimicrobial disk
- Ofloxacin 100 µg/ml - Amikacin 10000 µg/ml - Ampicillin 10000 µg/ml ↓ Change antibiotic disk by replacing of 40% glucose disk	- MG 1655 (positive; *E. coli*) - SRE94 (negative control) - SRE 12, 25, 78 (High persistent) - LF82_GPF, TRI 8, 9, 10 (mutant)	 - Tolerance strains:LF82_GPF, TRI 8, 9, 10  - High persistent:SRE 12, 25, 78

Figure 4. Colony count of *Escherichia coli* on agar plate and glass bead for spreading of bacteria.

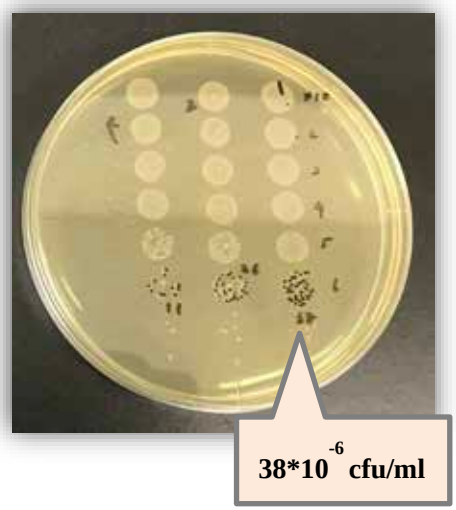
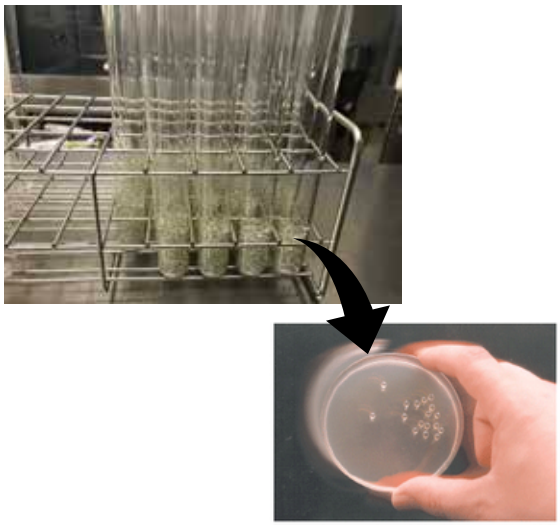
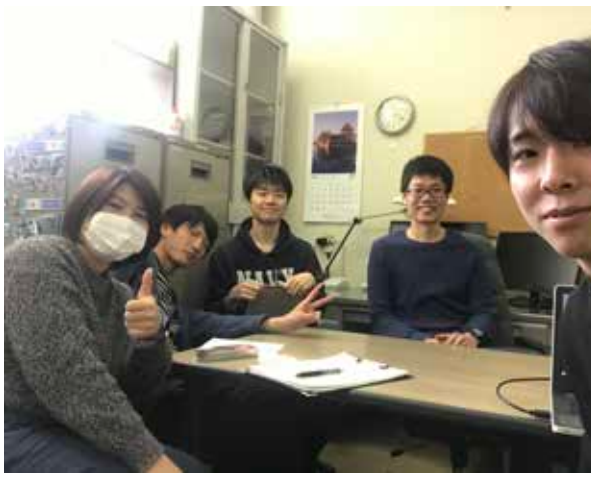
Colony count of *Escherichia coli* on LB agar plate	Glass bead for spreading of bacteria
	

Figure 5. Presentation for internship report activities and discussion.



- What do you think the positive impact of the activity will have on your further career path?

I would like to be a lecturer at the University in my country after graduation. This internship, I was able to see the teaching way of Dr. Usui to make undergraduate students understanding well. I think this is a great opportunity to learn the way how to teach students in various topics of antimicrobial resistance such as clinical, livestock, and environment. Moreover, I obtained many suggestions from Dr. Usui for my future research in bacterial infection fields. If I have a chance to collaborate with Dr. Usui in the future. And we may make a strong collaboration network between Thailand and Japan.

- Advice for your junior fellows

- Design your plan with your supervisor which institute you would go.
- Apply internship application three months before or if you need a visa to go aboard is needed to submit six months before departure.
- When faced with any problems, we should be confident and always thinking about how to solve problems.

Approval of supervisor	Institution・Official title・Name Division of Bioresources, Research Center for Zoonosis Control Professor Yasuhiko Suzuki

※1 Send the electronic file to the Leading School section, International Affairs Office
※2 Attach a copy certificate of the content of internship activity that is prepared by the counterpart at the internship institution (any form with a signature of the counterpart).
※3 The Steering Committee of the Leading Program will first confirm the content of this report and report will be forwarded to the Educational Affairs Committee for credits evaluation.

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