Overseas Practice on（Field Epidemiology・Collaborative Research）
report form（For Student）

2018/01/04 (Year/Month/Day)

| | |
|---|---|
| Name | Ruttana Pachanon |
| Laboratory | Bioresources |
| Year (Grade) | D2 |
| Place of practice | Faculty of Public Health, Thammasat University, Pathum Thani Province, Thailand |
| Period of practice | November 7th 2017 – December 15th 2017 |
| Purpose | Surveillance of *Escherichia coli* and *Salmonella* isolated from swine farms and pork in Central region of Thailand |

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

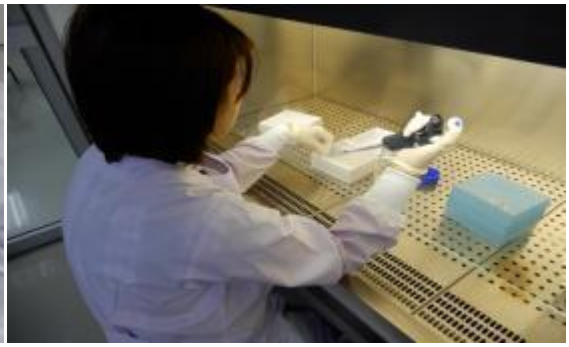
**Background and purpose**

Foodborne diseases caused by *E. coli* and *Salmonella* have been recognized as a major concern for public health. These bacteria can be transmitted from animal reservoirs to humans by consuming contaminated water/food and through direct contact with the animals. Swine is a one of a food-producing animal. Moreover, the antimicrobial agents have been frequently used in swine production for therapeutic, growth promotion purposes and preventing the diseases, especially in the development of multidrug-resistant bacteria. The previous study in Thailand found that 68-99% of drug resistance phenotypes from *E. coli* and *Salmonella* isolated in swine (Changkaew et al., 2015, Chotinun et al., 2014). It suggested that there was a high incidence of antimicrobial resistance (AMR). Therefore, we need to determine the prevalence of *E. coli* and *Salmonella* in swine and their antimicrobial resistance to provide information for the nationwide surveillance of AMR.

**My activity at Faculty of Public Health, Thammasat University, Thailand during 7 November -15 December 2017**

**November 7th - 10 th 2017**: I met the Dean, accompanying supervisor (Prof. Dr. Orasa Suthienkul) and investigator (Dr. Kanjana Changkaew) and then laboratory tour at Faculty of Public Health, Thammasat University. I have received a lecture about overview of conventional culture method for isolation and identification of *E. coli* and Nontyphoidal *Salmonella*. In the next day, culture media were prepared for isolation and identification of *E. coli* and *Salmonella*.

**November 13th - 24th 2017**: Culture media were prepared for isolation and identification of *E. coli* and *Salmonella.* The *E. coli* and *Salmonella* were isolated from 40 feces samples at Suphanburi province located in Central region of Thailand by conventional method.

**November 27th - 28th 2017:** Culture media were prepared for isolation and identification of *E. coli* and *Salmonella.*

**November 29th 2017:** Sample collection at Pathumthani market, Thailand

1. Collected pork and cutting board from 6 shops



Pork about 300 g  Pork on the surface of cutting board

2. Questionnaire survey by investigators

**November 29th to December 3th 2017:**

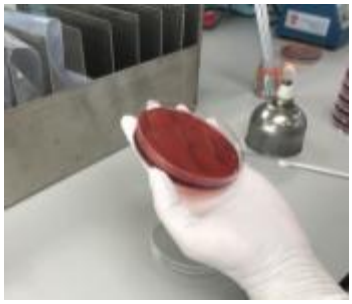
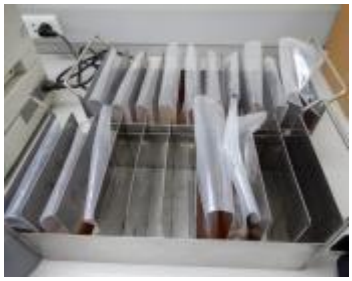
**Sample preparation and enrichment procedure**

1. Prepare 300 g of pork sample and pork on the surface of cutting board

2. Weigh 25 g of pork into 225 ml of brain heart infusion (BHI) broth for *E. coli* and 225 ml of buffered peptone water (BPW) for *Salmonella* and stomach briefly as necessary.



3. Isolation and identification of *E. coli* and *Salmonella* by conventional culture method

| ***E. coli*** | ***Salmonella*** |
|---|---|
| 1. Isolation of *E. coli*<br>● Direct method | 1. Isolation of *salmonella*<br>● Enrichment method |
|  |  |
| Streaked the sample onto Macconkey agar | Swab the pork into 10 ml of buffered peptone water and incubated the sample at 37C°, overnight |
|  |  |
| Incubated the sample at 37C°, overnight | Rappaport-Vassiloadis (RV) enrichment broth |
| ● Enrichment method |  |

2. Identification of *E. coli* by biochemical tests

Picked up of pink colonies on Macconkey agar

(approximately 1-5 colonies/sample)

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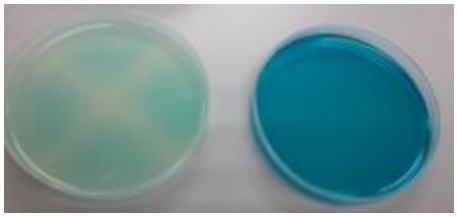
**IMViC tests**

2. Identification of *salmonella* by biochemical tests



Positive Negative

on Modified Semisolid Rappaport Vassiliadis

(MSRV) agar

Picked up of colerless colony and $HS_2$ in the center

on Xylose Lysine Deoxycholate agar

(approximately 1-5 colonies/sample)

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**TSI and LIM tests**

3. Interpretation of the result of biochemical tests

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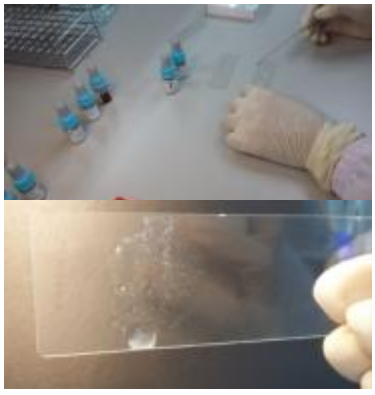
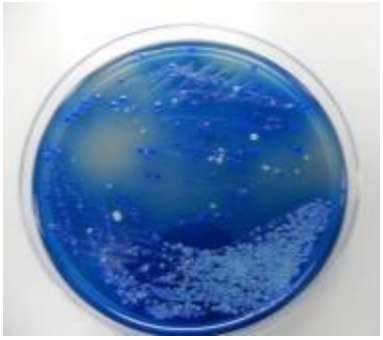
4. Serological test for serogrouping of *Salmonella* spp.



Positive for serological test

**Other identification (methicillin resistant *S. aureus*)**



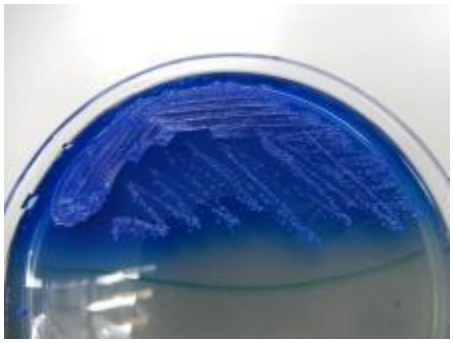
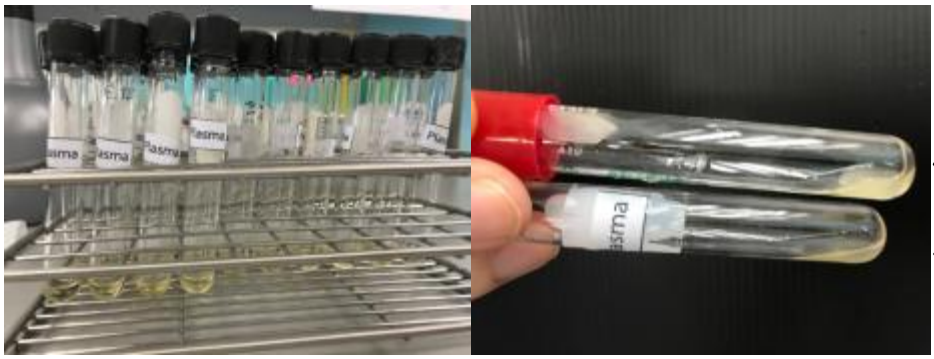
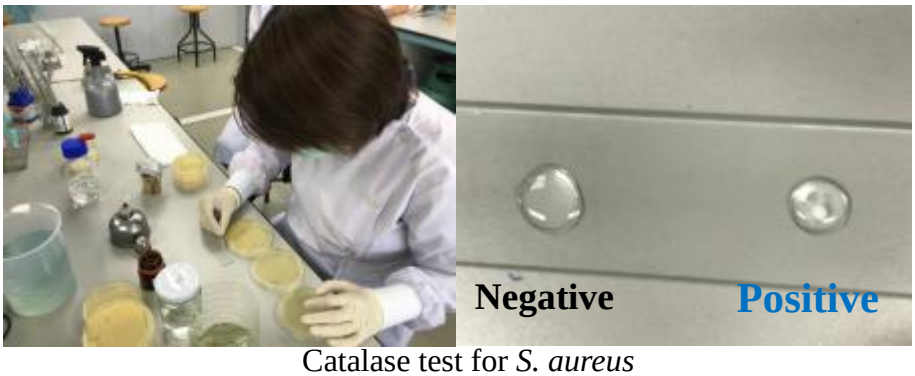


Plate from sample  Positive for *S. aureus* (Reference)

Blue colonies on oxacillin resistance screening agar base (ORSAB) plates




Coaglulase test for *S. aureus*




Catalase test for *S. aureus*

**December 4th 2017:** Attended a distinguished lecture by Prof. Dr. Hiroshi Kida

**December 6th – 7th 2017:**

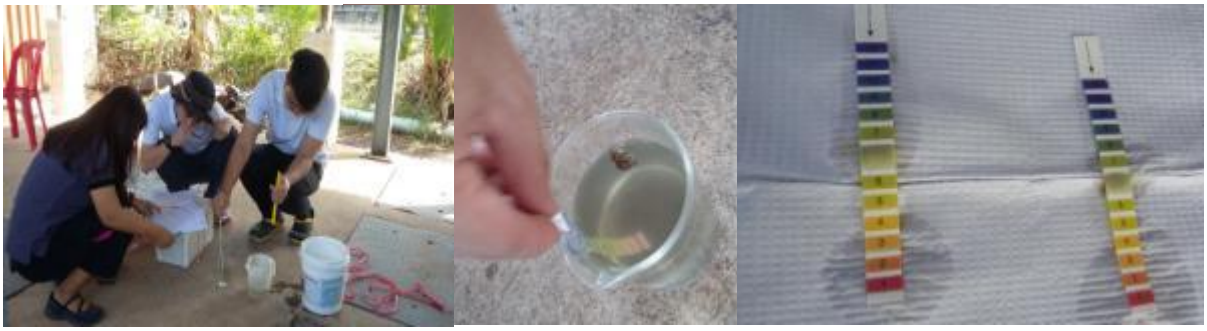
- Isolation and identification of *Salmonella* spp.
- Attended Mr. Kentaro Koide's presentation

**December 8th – 15th 2017:**

- Presentation of my research activity
- DNA extraction of the *E. coli* isolates by boiled method
- Testing PCR condition of *E. coli* reference strain

**Additional sample collection**

1. Waste water in area Thammasat University (November 23th 2017)



2. Air sampling for bacteria and fungi at Faculty of Medicine (November 28th 2017)

**Results**

- The prevalence of *E. coli* and *Salmonella* in swine from farms, pork and pork on surface of cutting board from markets as shown below;

| Source | Total of samples | No. positive of *E. coli* | | No. positive of *Salmonella* | |
|---|---|---|---|---|---|
| | | Samples | Isolates | Samples | Isolates |
| **Farm** (Feces) | 40 | 40 | 327 | 5 | 14 |
| **Market** | | | | | |
| - Pork | 6 | 6 | 41 | 4 | 7 |
| - Pork on surface of cutting board | 6 | 6 | 44 | 4 | 13 |

**Outcome**

- I have learned more about field epidemiology for sample collection in the market, isolation and identification of *E.coli* and *Salmonella* spp. including methicillin resistant *S. aureus* (MRSA).
- I have more skill of the serological test for serogrouping of *Salmonella* spp. and also molecular technique for DNA extraction and testing PCR condition.
- However, *Salmonella* serovar will be confirmed by *Salmonella* and *Shigella* Center, Nonthaburi Thailand. And also the team (investigators at Faculty of Public Health, Thammasat University), they need to further determine the antimicrobial resistant pattern of *E.coli* and *Salmonella*. In the future, I may be obtained opportunity from the collaborative at Faculty of Public Health, Thammasat University, Thailand.

（Field Epidemiology・Collaborative Research） Evaluation by supervisor

| Institution・Official title・Name | Prof. Yasuhiko Suzuki 印 |
|---|---|
| Describe overall evaluation on the applicant’s activity in overseas practice. | |