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

2015/06/10 (Year/Month/Day)

Name	SIRIPORN KONGSOI
Laboratory	Division of Bioresources, Hokkaido University Research Center for Zoonosis Control
Year (Grade)	4th year PhD student
Place of practice	Veterinary Diagnostic Service, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen District, Nakhon Pathom, Thailand, 73140
Period of practice	20 April – 22 May 2015
Purpose	Field epidemiology on the prevalence of mutations within the quinolone resistance-determining region of *gyrA*, *gyrB*, *parC*, and *parE* and association with antibiotic resistance in quinolone-resistant *Salmonella enterica* in diseased swine.

Salmonella spp. comprises one of the most important bacterial-zoonotic pathogens, causing acute food-borne diseases in humans, and is recognized as a major public health problem. Although contaminated eggs and raw or undercooked poultry are the primary sources of salmonellosis in humans, pork causes an estimated 15-20% of all cases. While contamination can occur during any process along the food production line, infected pigs on the farm are the origin of the contaminated pork that leads to human infections. Pig farmers routinely use antibiotics for both treatment and prophylactic purposes. Excessive and incorrect uses of antibiotics are probably a primary cause of increasing bacterial resistance. There is a concern for the development of resistant *Salmonella* that may pass to people through the food chain and can produce untreatable disease. For example, a *Salmonella* strain of pig origin with decreased susceptibility to quinolones caused a severe outbreak of salmonellosis in Denmark where two people died (Mølbak et al., 1999). In salmonellae, DNA gyrase is the primary target of quinolone action. A single point mutation in the quinolone resistance-determining region (QRDR) of *gyrA* can mediate resistance to the non-fluorinated quinolone nalidixic acid and reduced susceptibility to fluoroquinolones such as ciprofloxacin. Mutations in the gyrB and topoisomerase IV genes *parC* and *parE* are considered rare in salmonellae. It was hypothesized that those isolates with decreased susceptibility harbored a single mutation in gyrA, whereas resistant isolates would contain two or more mutations in *gyrA* and/or *gyrB* and/or *parC* and/or *parE*.

The objectives of this study were to characterize *Salmonella* spp. by phenotyping and serotyping characterizations. In addition, quinolones susceptibility profile and the presence of mutations in the QRDR of chromosomal *gyrA*, *gyrB*, *parC* and *parE* genes were assessed. The flow of study was shown in Figure 1.

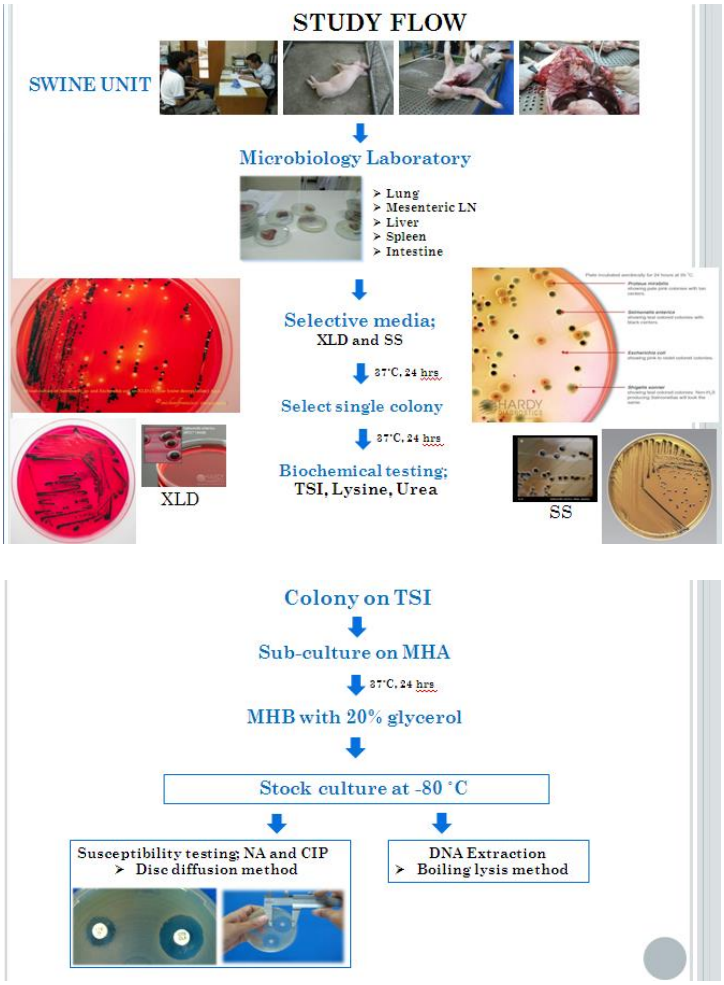


Figure 1. Study flow for isolation and identification, susceptibility testing and DNA extraction

Isolation and identification. *Salmonella* isolates from different swine organs were collected from 8 diseased swine, as shown in Figure 2, from Veterinary Diagnostic Service, Faculty of Veterinary Medicine, Kasetsart University during the 20 April – 22 May 2015. *Salmonella* isolates (n=91) were identified and confirmed to be *Salmonella* on the basis of biochemical reactions on triple sugar iron agar, urea and lysine. The summarized results were shown in Table 1.

Table 1. The summarized results of biochemical testing and susceptibility testing

Isolation no.	Organ origin	Selective media	Biochemical results			MIC				Stock no.
			TSI	Lysine	Urea	NA		CIP		
						mm.	Resistant phenotype	mm.	Resistant phenotype	
M272-XLD1	Intestine	XLD	K/A H_2S+	+	-	23	S	36	S	1
M272-XLD2	MSLN	XLD	K/A H_2S+	+	-	23	S	36	S	2
M272-XLD3	MSLN	XLD	K/A H_2S+	+	-	23	S	36	S	3
M272-XLD4	MSLN	XLD	K/A H_2S+	+	-	22	S	35	S	4
M272-XLD5	MSLN	XLD	K/A H_2S+	+	-	25	S	35	S	5
M272-XLD6	MSLN	XLD	K/A H_2S+	+	-	24	S	36	S	6
M272-SS7	MSLN	SS	K/A H_2S+	+	-	22	S	36	S	7
M272-SS8	MSLN	SS	K/A H_2S+	+	-	22	S	36	S	8
M272-SS9	MSLN	SS	K/A H_2S+	+	-	22	S	35	S	9
M272-SS10	MSLN	SS	K/A H_2S+	+	-	22	S	36	S	10
M272-SS11	MSLN	SS	K/A H_2S+	+	-	23	S	35	S	11
M284-XLD1	Lung	XLD	K/A H_2S-	+	-	0	R	20	R	12
M284-XLD2	Lung	XLD	K/A H_2S-	+	-	0	R	20	R	13
M284-XLD3	Lung	XLD	K/A H_2S-	+	-	0	R	20	R	14
M284-XLD4	Lung	XLD	K/A H_2S-	+	-	0	R	20	R	15
M284-XLD5	Lung	XLD	K/A H_2S-	+	-	0	R	20	R	16
M284-SS6	Lung	SS	K/A H_2S-	+	-	0	R	17	R	17
M284-SS7	Lung	SS	K/A H_2S-	+	-	0	R	18	R	18
M284-SS8	Lung	SS	K/A H_2S-	+	-	0	R	20	R	19
M284-SS9	Lung	SS	K/A H_2S-	+	-	0	R	20	R	20
M284-SS10	Lung	SS	K/A H_2S-	+	-	0	R	18	R	21
M284-XLD11	Spleen	XLD	K/A H_2S-	+	-	0	R	18	R	22
M284-XLD12	Spleen	XLD	K/A H_2S-	+	-	0	R	19	R	23
M284-XLD13	Spleen	XLD	K/A H_2S-	+	-	0	R	18	R	24
M284-XLD14	Spleen	XLD	K/A H_2S-	+	-	0	R	17	R	25
M284-XLD15	Spleen	XLD	K/A H_2S-	+	-	0	R	19	R	26

Isolation no.	Organ origin	Selective media	Biochemical results			MIC				Stock no.
			TSI	Lysine	Urea	NA		CIP		
						mm.	Resistant phenotype	mm.	Resistant phenotype	
M284-XLD16	Spleen	SS	K/A H_2S-	+	-	0	R	19	R	27
M284-XLD17	Spleen	SS	K/A H_2S-	+	-	0	R	19	R	28
M284-XLD18	Spleen	SS	K/A H_2S-	+	-	0	R	20	R	29
M284-XLD19	Spleen	SS	K/A H_2S-	+	-	0	R	19	R	30
M284-XLD20	Spleen	SS	K/A H_2S-	+	-	0	R	18	R	31
M286-XLD1	Lung	XLD	K/A H_2S-	+	-	0	R	19	R	32
M286-XLD2	Lung	XLD	K/A H_2S-	+	-	0	R	18	R	33
M286-XLD3	Lung	XLD	K/A H_2S-	+	-	0	R	21	I	34
M286-XLD4	Lung	XLD	K/A H_2S-	+	-	0	R	19	R	35
M286-XLD5	Lung	XLD	K/A H_2S-	+	-	0	R	19	R	36
M286-SS6	Lung	SS	K/A H_2S-	+	-	0	R	20	R	37
M286-SS7	Lung	SS	K/A H_2S-	+	-	0	R	19	R	38
M286-SS8	Lung	SS	K/A H_2S-	+	-	0	R	18	R	39

Isolation no.	Organ origin	Selective media	Biochemical results			MIC				Stock no.
			TSI	Lysine	Urea	NA		CIP		
						mm.	Resistant phenotype	mm.	Resistant phenotype	
M286-SS9	Lung	SS	K/A H_2S-	+	-	0	R	20	R	40
M286-SS10	Lung	SS	K/A H_2S-	+	-	0	R	20	R	41
M287-XLD1	Spleen	XLD	K/A H_2S-	+	-	0	R	20	R	42
M287-XLD2	Spleen	XLD	K/A H_2S-	+	-	0	R	20	R	43
M287-XLD3	Spleen	XLD	K/A H_2S-	+	-	0	R	19	R	44
M287-XLD4	Spleen	XLD	K/A H_2S-	+	-	0	R	19	R	45
M287-XLD5	Spleen	XLD	K/A H_2S-	+	-	0	R	18	R	46
M287-SS6	Spleen	SS	K/A H_2S-	+	-	0	R	17	R	47
M287-SS7	Spleen	SS	K/A H_2S-	+	-	0	R	20	R	48
M287-SS8	Spleen	SS	K/A H_2S-	+	-	0	R	20	R	49
M287-SS9	Spleen	SS	K/A H_2S-	+	-	0	R	19	R	50
M287-SS10	Spleen	SS	K/A H_2S-	+	-	0	R	20	R	51
M415-XLD1	Lung	XLD	K/A H_2S+	+	-	18	I	24	I	52

Isolation no.	Organ origin	Selective media	Biochemical results			MIC				Stock no.
			TSI	Lysine	Urea	NA		CIP		
						mm	Resistant phenotype	mm	Resistant phenotype	
M415-XLD2	Lung	XLD	K/A H_2S+	+	-	17	I	24	I	53
M415-XLD3	Lung	XLD	K/A H_2S+	+	-	18	I	25	I	54
M415-XLD4	Lung	XLD	K/A H_2S+	+	-	17	I	23	I	55
M415-XLD5	Lung	XLD	K/A H_2S+	+	-	17	I	23	I	56
M415-SS6	Lung	SS	K/A H_2S+	+	-	18	I	27	I	57
M415-SS7	Lung	SS	K/A H_2S+	+	-	17	I	26	I	58
M415-SS8	Lung	SS	K/A H_2S+	+	-	17	I	22	I	59
M415-SS9	Lung	SS	K/A H_2S+	+	-	18	I	26	I	60
M415-SS10	Lung	SS	K/A H_2S+	+	-	18	I	27	I	61
M457-XLD1	Intestine	XLD	K/A H_2S+	+	-	17	I	22	I	62
M457-XLD2	Intestine	XLD	K/A H_2S+	+	-	18	I	23	I	63
M457-XLD3	Intestine	XLD	K/A H_2S+	+	-	18	I	23	I	64
M457-XLD4	Intestine	XLD	K/A H_2S+	+	-	17	I	23	I	65

Isolation no.	Organ origin	Selective media	Biochemical results			MIC				Stock no.
			TSI	Lysine	Urea	NA		CIP		
						mm	Resistant phenotype	mm	Resistant phenotype	
M457-XLD5	Intestine	XLD	K/A H_2S+	+	-	18	I	23	I	66
M457-SS6	Intestine	SS	K/A H_2S+	+	-	18	I	22	I	67
M457-SS7	Intestine	SS	K/A H_2S+	+	-	18	I	23	I	68
M457-SS8	Intestine	SS	K/A H_2S+	+	-	18	I	21	I	69
M462-XLD8	MSLN	XLD	K/A H_2S-	+	-	0	R	19	R	70
M462-XLD9	MSLN	XLD	K/A H_2S-	+	-	0	R	18	R	71
M462-XLD10	MSLN	XLD	K/A H_2S-	+	-	0	R	18	R	72
M462-XLD11	MSLN	XLD	K/A H_2S-	+	-	0	R	20	R	73
M462-XLD12	MSLN	XLD	K/A H_2S-	+	-	0	R	20	R	74
M462-SS1	MSLN	SS	K/A H_2S+	+	-	18	I	28	I	75
M462-SS2	MSLN	SS	K/A H_2S+	+	-	18	I	30	I	76
M462-SS3	MSLN	SS	K/A H_2S-	+	-	0	R	19	R	77
M462-SS4	MSLN	SS	K/A H_2S-	+	-	0	R	19	R	78

Isolation no.	Organ origin	Selective media	Biochemical results			MIC				Stock no.
			TSI	Lysine	Urea	NA		CIP		
						mm	Resistant phenotype	mm	Resistant phenotype	
M462-SS5	MSLN	SS	K/A H_2S-	+	-	0	R	19	R	79
M462-SS6	MSLN	SS	K/A H_2S-	+	-	0	R	19	R	80
M462-SS7	MSLN	SS	K/A H_2S-	+	-	0	R	20	R	81
M463-XLD1	Lung	XLD	K/A H_2S-	+	-	0	R	20	R	82
M463-XLD2	Lung	XLD	K/A H_2S-	+	-	0	R	20	R	83
M463-XLD3	Lung	XLD	K/A H_2S-	+	-	0	R	20	R	84
M463-XLD4	Lung	XLD	K/A H_2S-	+	-	0	R	20	R	85
M463-XLD5	Lung	XLD	K/A H_2S-	+	-	0	R	20	R	86
M463-SS1	Lung	SS	K/A H_2S-	+	-	0	R	20	R	87
M463-SS2	Lung	SS	K/A H_2S-	+	-	0	R	18	R	88
M463-SS3	Lung	SS	K/A H_2S-	+	-	0	R	18	R	89
M463-SS4	Lung	SS	K/A H_2S-	+	-	0	R	20	R	90
M463-SS5	Lung	SS	K/A H_2S-	+	-	0	R	20	R	91

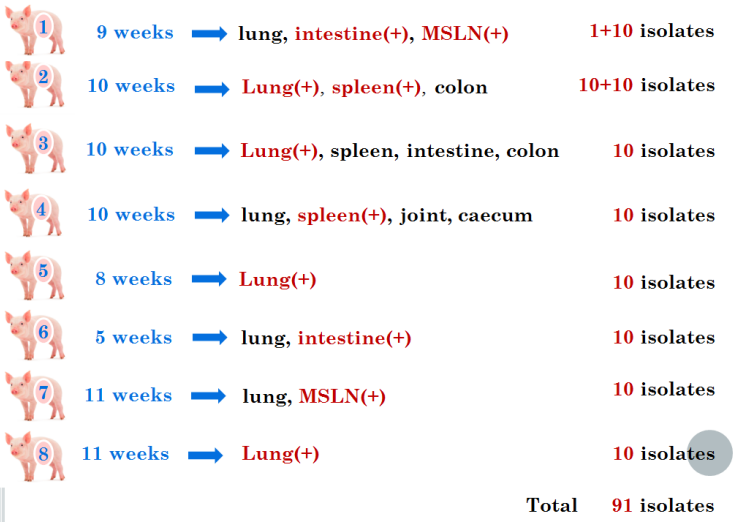
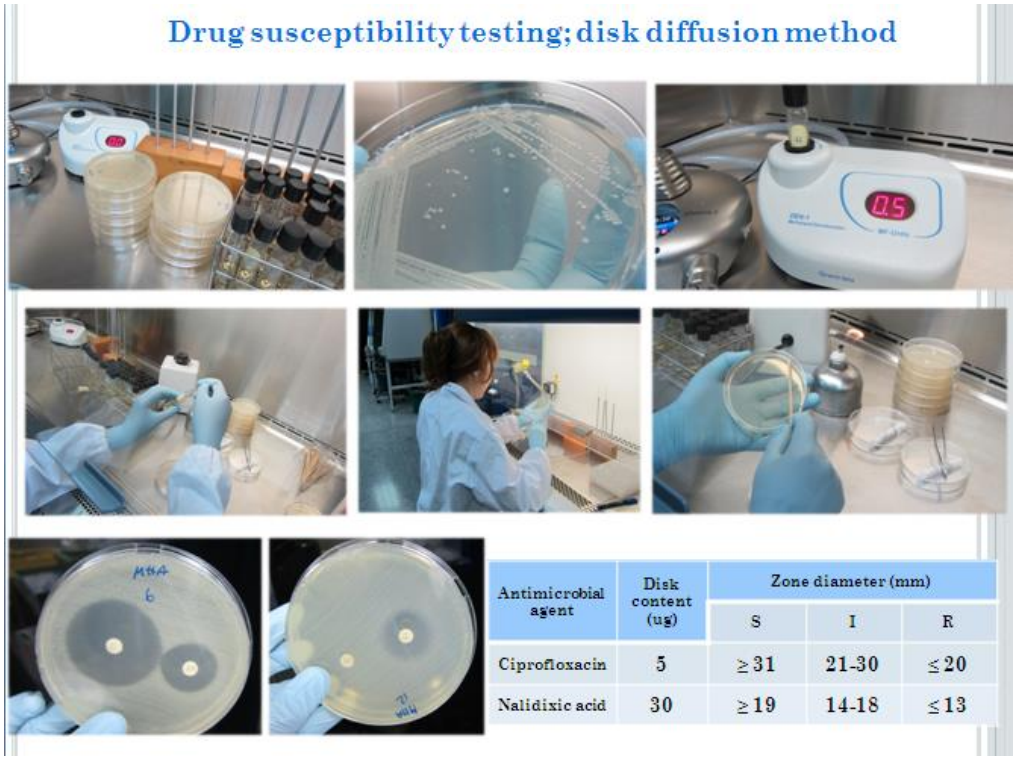


Figure 2. The total number of isolates was collected from 8 diseased swine in different organs.

Quinolones susceptibility testing. Susceptibility testing was performed by the disk diffusion method according to CLSI standards (Clinical and Laboratory Standards Institute, M31-A3, ANONYM., 2012) for the following antimicrobial agents: nalidixic acid (30 ug) and ciprofloxacin (5ug). *E. coli* ATCC 25922, was used as control strains (Figure3). Figure 4 and Figure 5 shows the susceptibility pattern of nalidixic acid and ciprofloxacin, respectively.



Antimicrobial agent	Disk content (ug)	Zone diameter (mm)		
		S	I	R
Ciprofloxacin	5	≥ 31	21-30	≤ 20
Nalidixic acid	30	≥ 19	14-18	≤ 13

Figure 3. Disk diffusion method and the criteria for interpretive the susceptibility pattern.

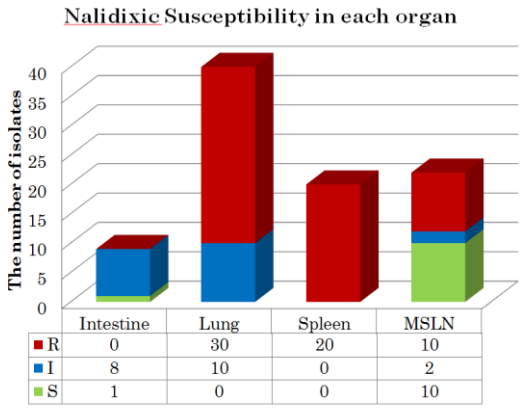
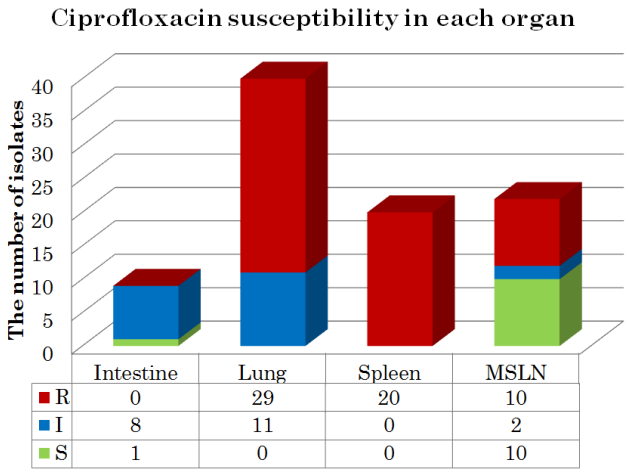


Figure 4. Nalidixic susceptibility pattern in *Salmonella* isolates from each organ (R-resistant, I-intermediate and S-susceptible).



	Intestine	Lung	Spleen	MSLN
R	0	29	20	10
I	8	11	0	2
S	1	0	0	10

Figure 5. Ciprofloxacin susceptibility pattern in *Salmonella* isolates from each organ (R-resistant, I-intermediate and S-susceptible).

DNA extraction. DNA extraction using centrifugation and boiling method, cell pellets were resuspended in 0.1 ml of TE buffer and were centrifuged at 15,000g for 10 min. Pellets were resuspended in 40 µl of TE buffer, subjected to boiling at 100°C in heat box for 10 min, cooled on ice, centrifuged at 15,000g for 10 s, and stored at -20°C until used.

（Field Epidemiology・Collaborative Research） Evaluation by supervisor

Institution・Official title・Name	印 Professor, Research Center for Zoonosis Control
Enough number of isolates of *Salmonella species* was obtained by Ms. Kongsoi's hard working. The data on the quinolone resistance might contribute to the control of salmonellosis among pigs.	

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