Scientific methods

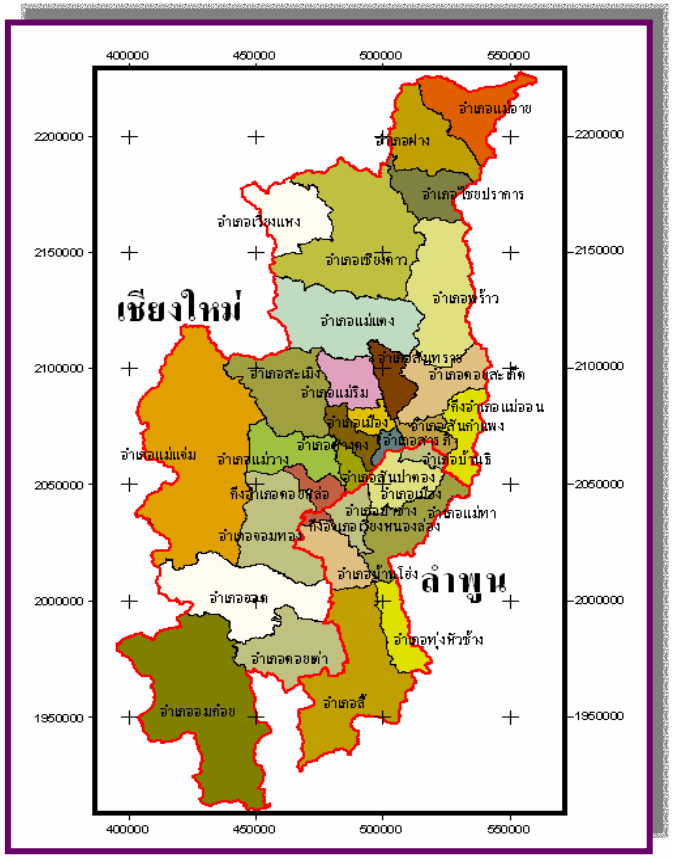
- Microbiology
  - Focus on individual sample
  - Demonstrate various mechanism in a small number of isolates
- Epidemiology
  - Focus on a population
  - Demonstrate a single mechanism in a large number of isolates

Objectives

- Determine the prevalence of class1 integrons in *E. coli* and *Salmonella* isolated from food animals in Thailand
- Determine the relationship between class1 integrons and antimicrobial resistance
- Characterize class1 integrons identified

Bacterial isolates

E. coli	Food Animal	258
	Human	6
	Farm env	26
	Total	**290**
Salmonella	Food Animal	117
	Human	0
	Farm env	167
	Total	**284**

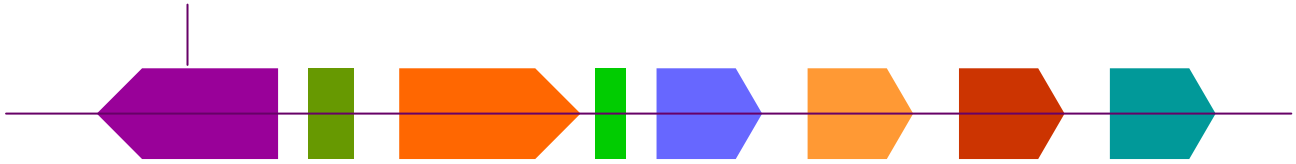


Disk diffusion susceptibility test (zone size; mm)

Antimicrobial agents	*E. coli* 25922	*S. aureus* 25923	Resistance Breakpoint
Ceftiofur (30 µg)	26-31	27-31	≤17
Chloramphenicol (30 µg)	21-27	19-26	≤12
Enrofloxacin (5 µg)	32-40	27-31	≤16
Erythromycin (15 µg)	-	22-30	≤13
Gentamicin (10 µg)	19-26	19-27	≤12
Oxacillin (1µg)	-	18-24	≤10
Trimethoprim-sulfamethoxazole	15-23	24-34	≤10
Tetracycline (30 µg)	18-25	24-30	≤14

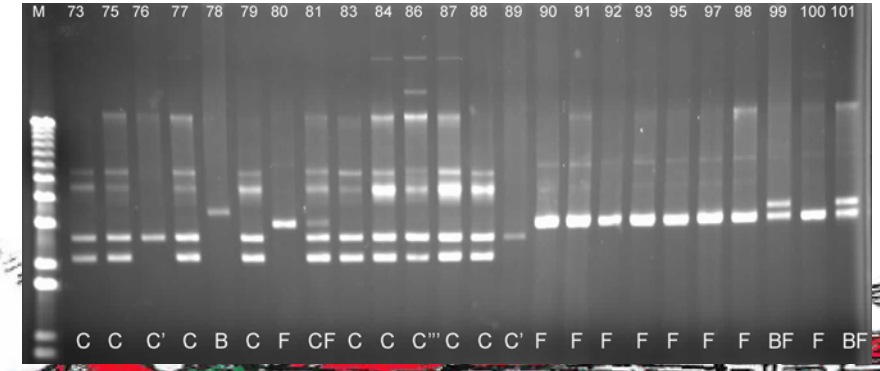
Integron characterization

- PCR - *intI*

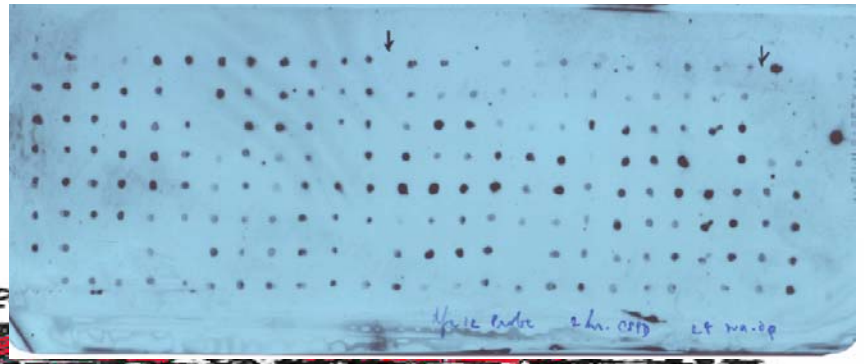


Resistance gene cassette

PCR and plasmid profiling



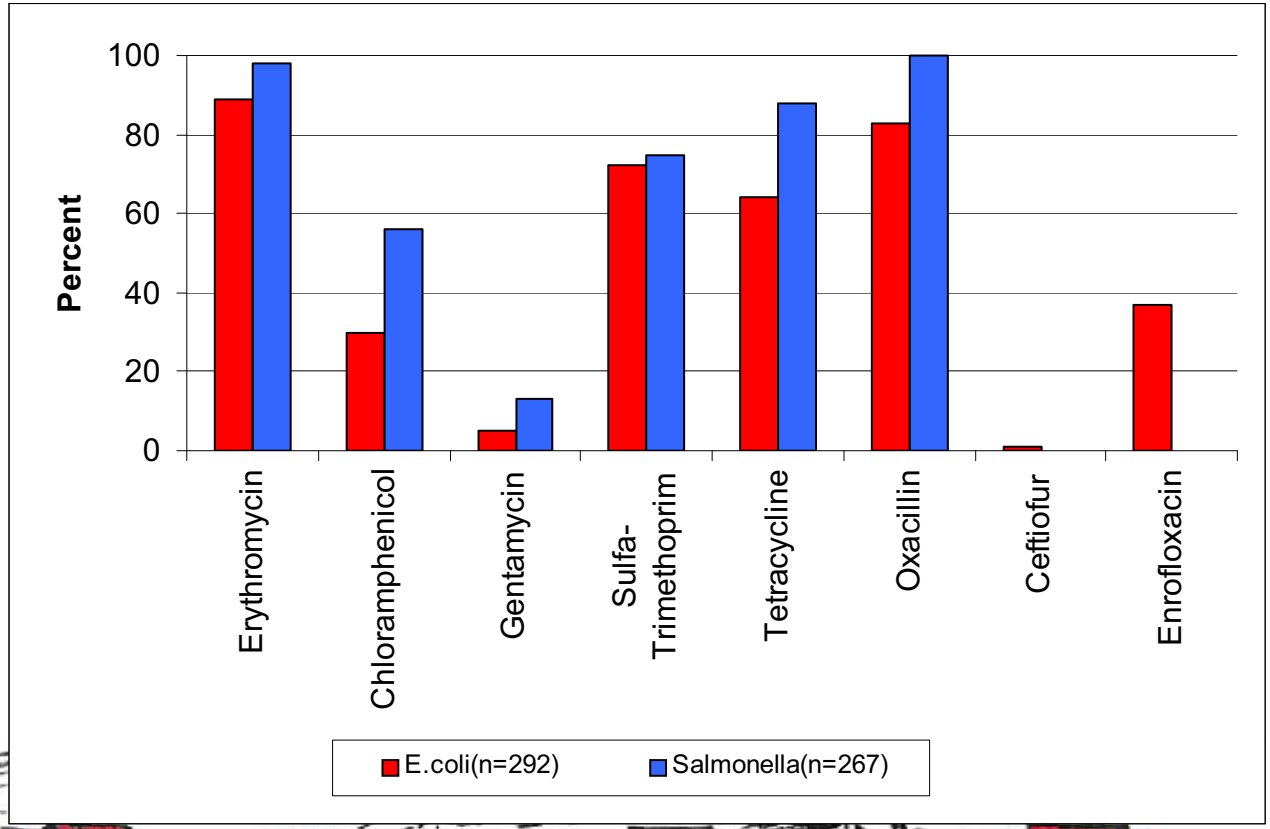
Dot-blot hybridization

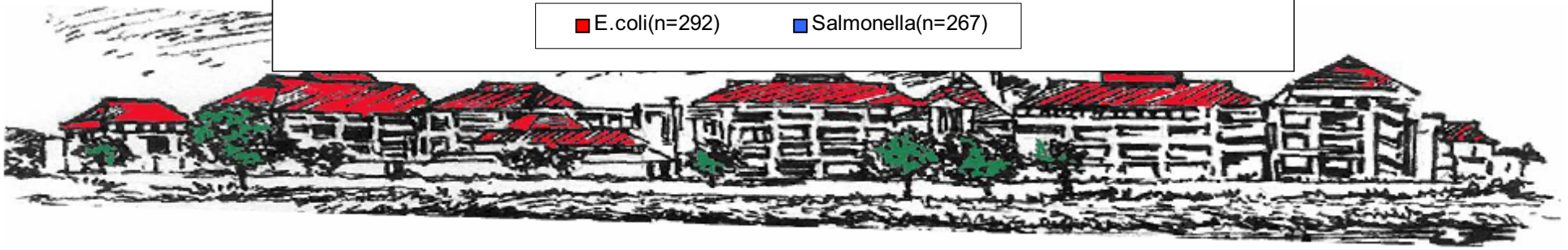


Statistical analysis

- Prevalence
  - Number of positive / number tested
- Multi-resistance
  - Resistance to more than 2 agents
- Relationship between integrons and multi-resistance
  - Fisher's exact test of independence
  - Mantel-Haenzel test for homogeneity of OR

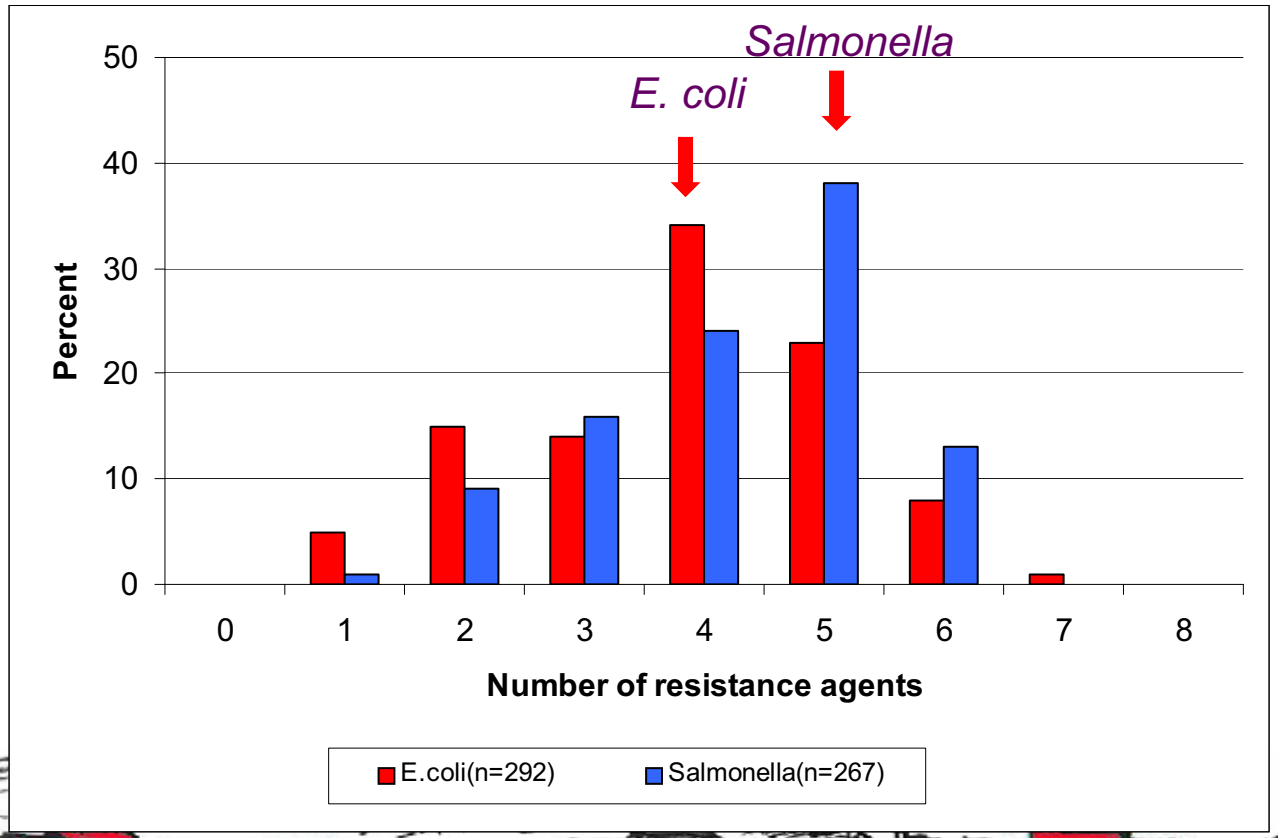
Results
Prevalence of antimicrobial resistance

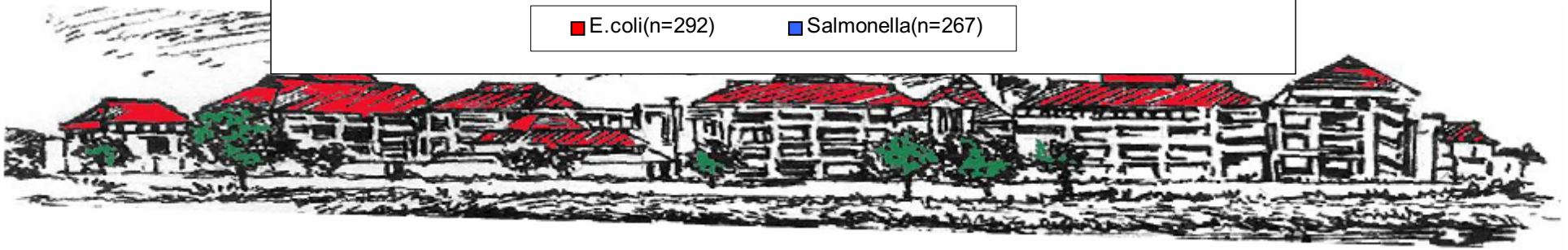




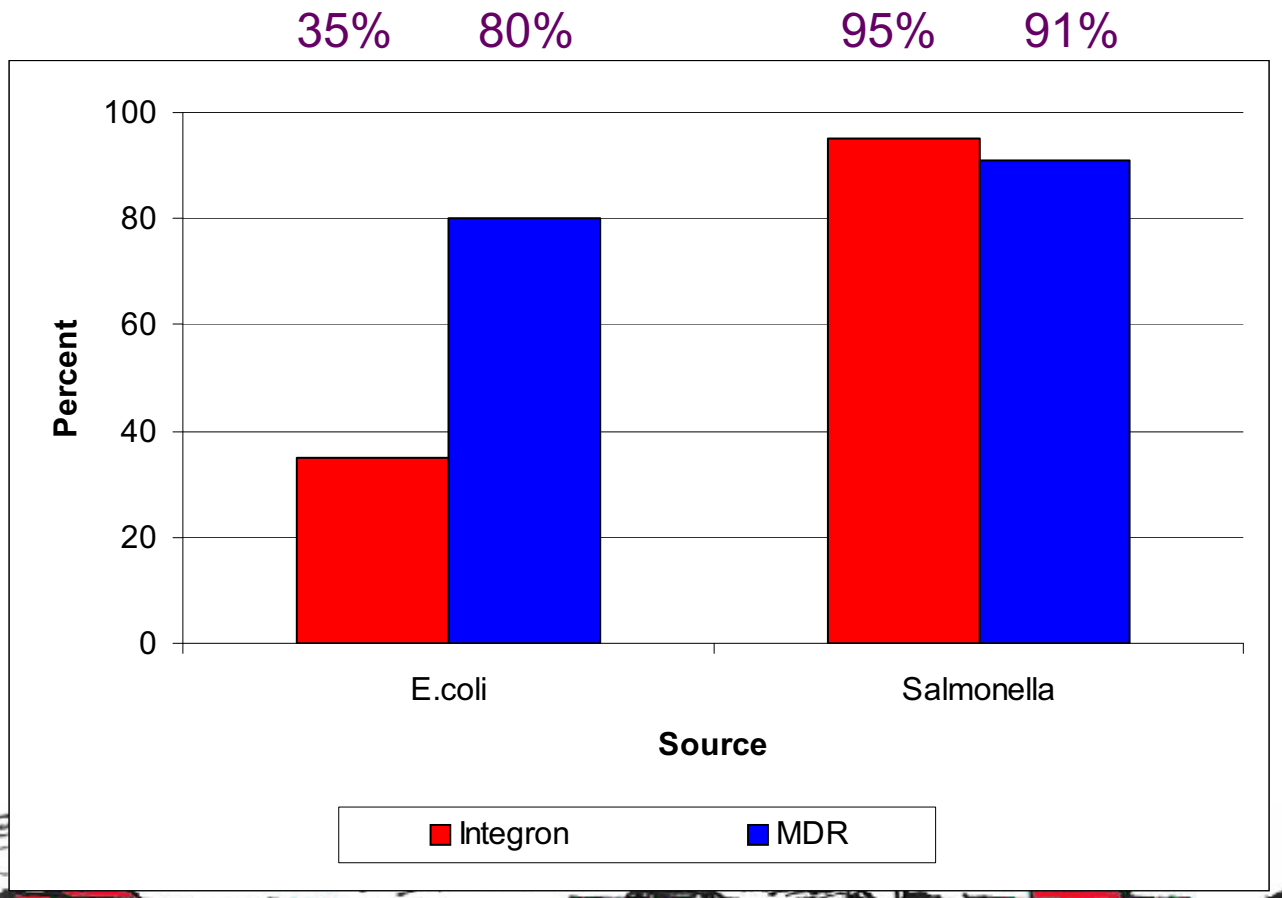
Results
Number of agents with resistance

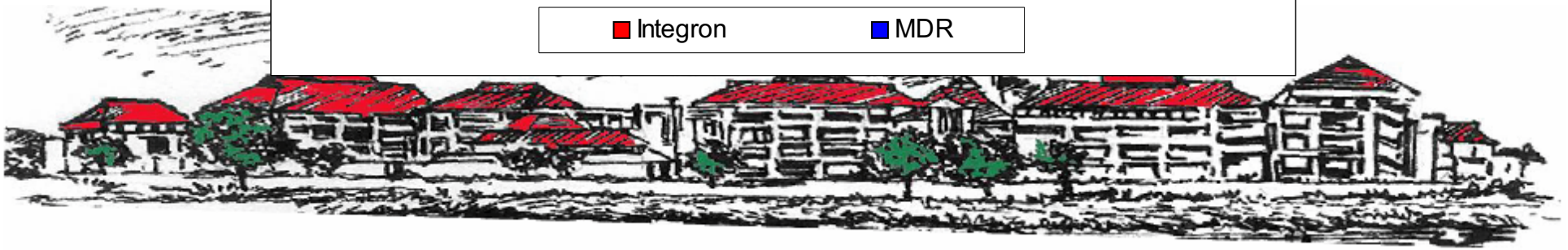
Median





Results
Prevalence of class1 integrons and MDR



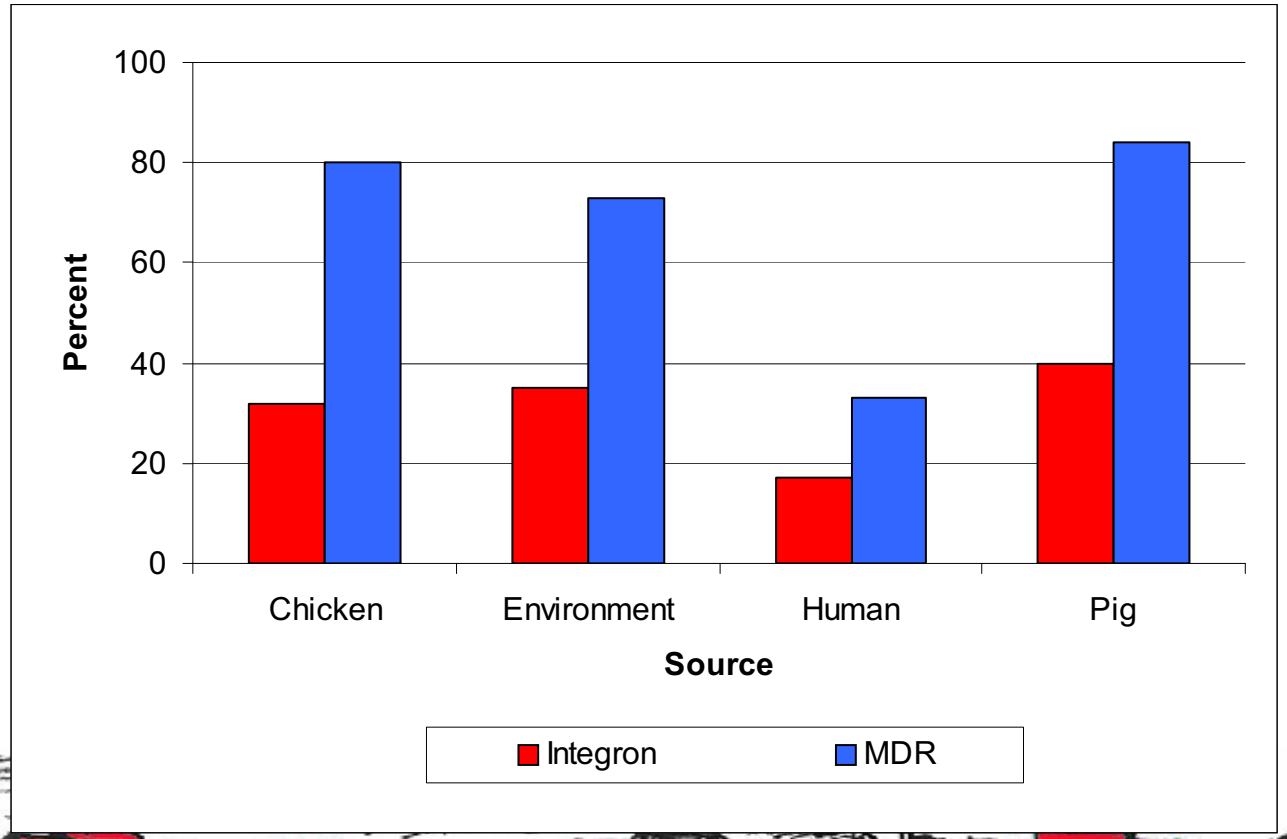


Result
Relationship between integrons and MDR

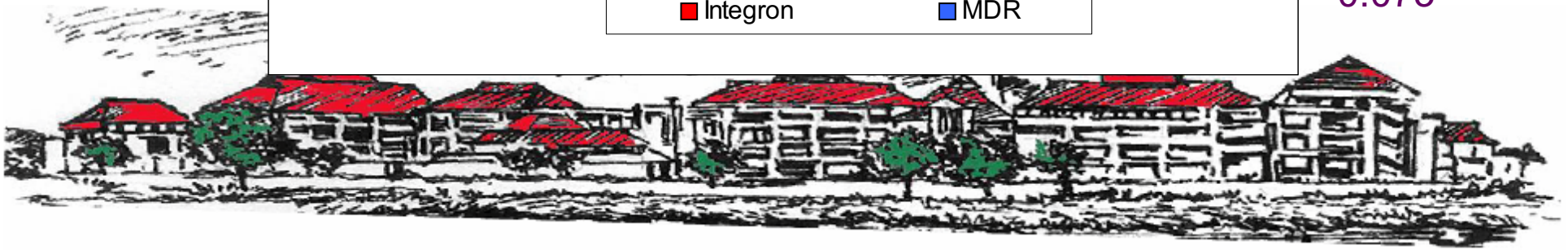
Bacteria	% INT/MDR	% MDR/INT	p
E.coli	80	88	0.014
Salmonella	97	93	0.006

Results
E. coli integrons and MDR by source

P = 0.558 P = 0.058 P = 1.000 P = 0.022

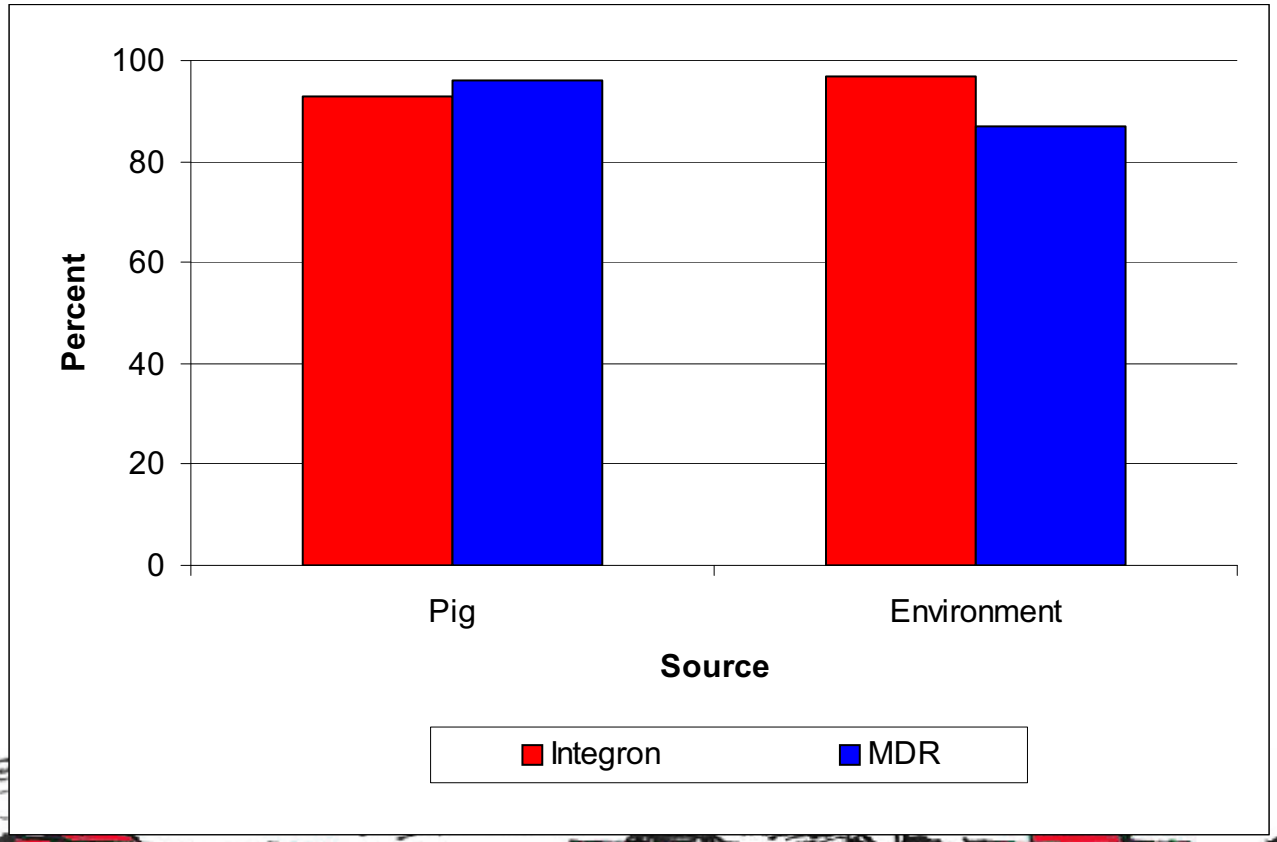


MH-p
0.073

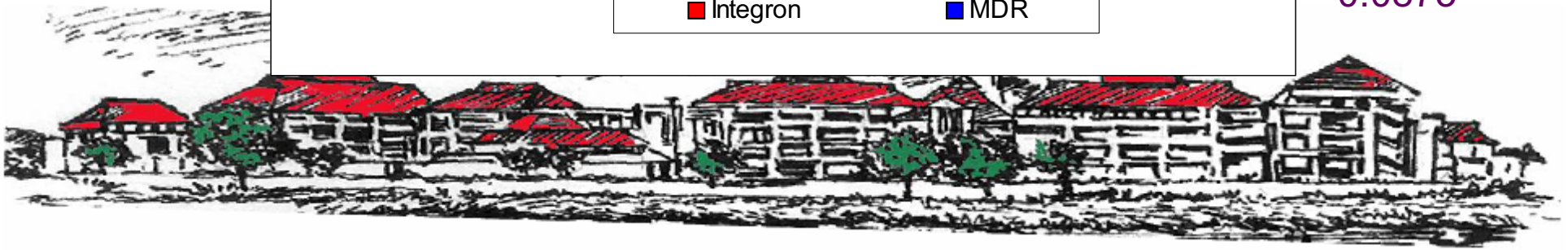


Results
Salmonella integrons and MDR by source

P = 0.029 P = 0.038



MH-p
0.0873



Results
Salmonella serovar

Serovar	n	%	% integrons	% MDR	% MDR/INT
Rissen	138	49	100	93	93
Typhimurium	54	19	97	100	100
Stanley	34	12	88	88	87
Others	55	20	40	100	89
				MH – p	0.877

Results
Salmonella serogroup

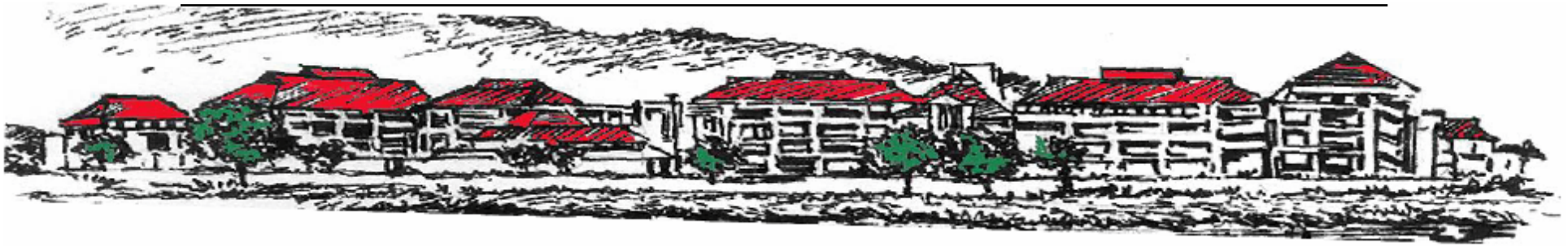
Serogroup	n	%	% integrons	% MDR	% MDR/INT
B	96	34	94	96	95
C	139	49	100	93	93
D	6	2	100	83	100
E	43	15	71	74	86
				MH – p	0.971

Results
Integron size (%)

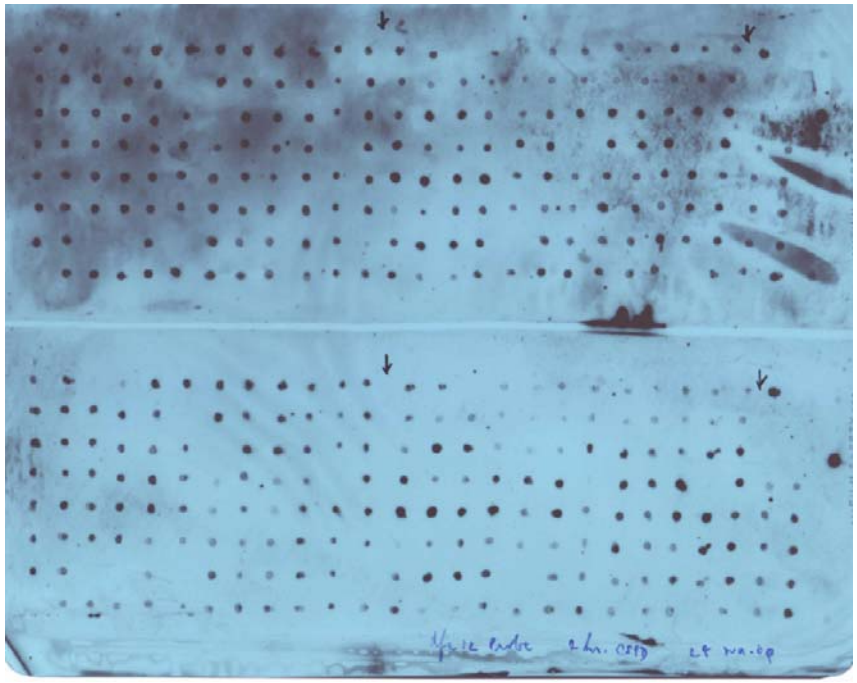
Size (kb)	*E. coli* (n=34)	*Salmonella* (n=79)
0.8	5.88	.
1	5.88	3.80
1.4	5.88	.
1.5	20.59	.
1.9	.	72.15
2	52.94	.
2.5	8.82	.

Results
Resistance gene in *E. coli* integrons

	n	%
aacA4, catB3, dfrA1	1	3
aadA2, Lnuf	2	6
aadA23	1	3
dfr17, aadA5	5	14
dfrA1, aadA1	5	14
dfrA12-aadA2	**16**	**46**
dfrA17-aadA5	1	3
dfrV	2	6
sat-sch_054-aadA2	2	6



Results
Resistance gene in *Salmonella* integrons



- *dfrA12, aadA2*
  - 196/197 positive

Result

Relationship between gene and resistance

Bacteria	gene	abx	% Resistance with gene	p
E. coli	*aad*	Gen	6.25	0.884
	dfr	Stx	83.3	0.512
Salmonella	*aad*	Gen	15.2	0.222
	dfr	Stx	78.3	0.849

Discussion

- Class1 integrons in *Salmonella* (95%)

Year	Place	Host	Percent
2008	Thailand	Pig & Chicken	25.6
2008	Spain	Pig	73.1
2007	Ethiopia	Food animal	53.1
2007	Canada	Broiler	0

Discussion

- Class1 integrons in *E. coli* (35%)

Year	Place	Host	Percent
2008	Thailand	Healthy Human	51
2008	Thailand	Patients	99
2007	Thailand	Market pigs	56.8
		Diarrheal pig	83.9
2005	China	Calf	59

Discussion

- Class1 integrons were associated with multi-drug resistance

Year	Place	Bacteria/host	%
2008	Thailand	*E. coli*/human	89 (MDR/INT)
2007	Thailand	*E. coli*/pig	100 (MDR/INT)
2007	Nepal	*Salmonella*/human	71 (INT/MDR)
2007	Ethiopia	*Salmonella*/animal	53 (INT/MDR)

Discussion

- *dfrA12* -*aadA2* are common resistance gene cassette in Salmonella
  - Taiwan, Ethiopia
- *dfrA17* -*aadA5* are second most common in *E. coli*
  - China
- Possession of resistance gene cassette was NOT associated with resistance to target agent
  - Other mechanisms
  - Silent gene

The next step

- What mechanism confer resistance in bacteria in food animal?
  - Source
    - Selection pressure during growth
    - Environment
  - Transmission
- How much does resistance gene transferred through food chain cost?
  - Quantitative risk assessment

Acknowledgement

- Center for Comparative Epidemiology, Michigan State University
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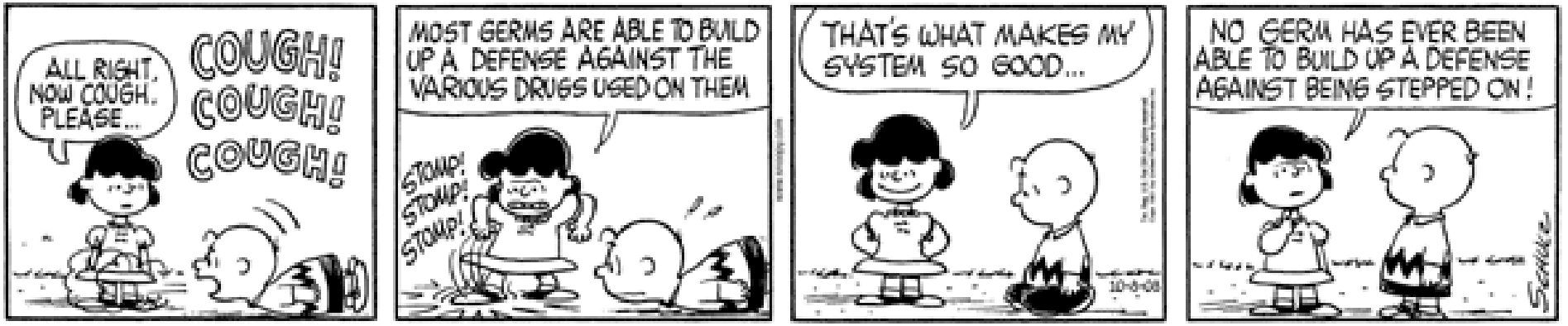
- Thai Research Fund (TRG4980001)

Conclusion

- Transferable resistance gene is common in food animal production environment
  - Increase virulence of food borne pathogen
  - Increase virulence of other pathogen
- Contribution of transferable resistance genes to resistance to antimicrobial agent in food require further investigation

Originally published Oct 11, 1961



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