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Original article

Prevalence and antimicrobial resistance of *Salmonella* serovars in chicken farm, Chiang Mai and Lamphun province, Northern of Thailand

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Abstract The study was conducted to clarity the prevalence of serovars and antimicrobial resistance in *Salmonella* isolates from chicken and environment in 24 chicken farms in Chiang Mai and Lamphun province, Thailand. Thirty seven (12.9%, 95%CI=9.0-16.7) and twenty five (52.1%, 95%CI=37.4-66.7) *Salmonella* isolates were found from 288 chickens and 48 environment samples, respectively. *Salmonella* isolates were found from chicken and environment in the first sampling were 28 (19.4%, 95% CI=12.9-26.0 and 8 (33.3. 95%CI=13.0-53.6), respectively. The most frequently *Salmonella* spp. found in chickens were *Salmonella* Corvalis, *Salmonella* Albany, *Salmonella* Enteritidis, and *Salmonella* Virchow. In contrast, *Salmonella* Virchow was commonly found in environment. The antimicrobial resistance test showed resistance to ampicillin in both groups. The most common pattern of the resistance was the single resistant for ampicillin. Multiple resistances to ampicillin, tetracycline, streptomycin and chloramphenicol were found but the prevalence was low (4%). In conclusion, This study indicated resistance *Salmonella* contamination is the problem in the chicken farms. Regulation of antimicrobial use should be implemented by the authorities. Also, the surveillance system of antimicrobial use should be done in Thailand. **Chiang Mai Veterinary Journal 2014; 12(2): 85-93**

Keywords: Antimicrobial resistance, chicken, Chiang Mai, Lamphun, *Salmonella*

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Introduction

Salmonella is one of the foodborne pathogens and cause of salmonellosis disease. In the United States of America, more than 40,000 cases of salmonellosis per year are reported (CDC, 2010). Also, salmonellosis outbreaks were reported In Thailand (Wathee et al. 2012; Krit, 2013). There are many different serovars or serotypes of *Salmonella*. At the present, more than 2500 serovars are identified. *Salmonella* Typhimurium and *Salmonella* Enteritidis are the most common in the United States (CDC, 2010). While, the most common serovars in Thailand gastroenteritis patients were *Salmonella* Weltevreden, *Salmonella* Stanley, *Salmonella* Anatum, and *Salmonella* Rissen (Sirichote et al. 2010).

As a result of evolutionary pressure for bacteria to survive in the environment with increased use of antimicrobials for humans and animals, antimicrobial resistance has become a global public problem leading to treatment failure and severity of infection (WHO, 1997). There has been a large amount of poultry production in Thailand. In 2012, there were approximately 142 million chickens (Office of Agricultural Economics, 2012). Antimicrobial use is known to be heavy. The amount of antimicrobials used in animals in1998 was approximately USD 23 million (Thai Drug Control Division, 2003). From previous study, antimicrobial use in broiler farms in Thailand is very common. The purpose of antimicrobial use is enhancing animal production (Na Lampang et al., 2007). Although, there were many reports of prevalence of serovars and antimicrobial resistance of *Salmonella* of chicken in many countries such as Cambodia (Lay et al., 2011), Vietnam (An et al., 2010) and Thailand (Pathanasophon et al., 2007). but the prevalence were difference levels. So, the study of prevalence of Salmonella serovars and their antimicrobial resistance is necessary for revealing the present situation. The objectives of this study were 1) to clarify the *Salmonella* serovars and, 2) to examine the prevalence and pattern of antimicrobial resistance in Chiang Mai and Lamphun Province, northern of Thailand.

Materials and methods

Sample and sample size

The sample size was calculated from the prevalence of *Salmonella* spp. in broiler farm in Chaing Mai was 25% with a 95% confidence interval covering an error of 5 percentage points from the true value. Therefore, 288 samples were collected from 24 broiler farms in Chiang Mai and Lamphun province. Farms were selected with convenience technique. Six cloacal swabs from chicken and an environment samples were collected per farm in May to September 2009. Each farm was visited two times, at 1-7 days age of broiler and before slaughter. Fourty-eight environment sample was obtained from wearing cotton sock walking through bedding around the house. All cloacal swabs were kept in transport media and cotton socks were kept in plastic bag. All samples were stored at 4-8 °C and cultured within 24 hours.

Salmonella isolation and identification

Cloacal swab and cotton sock were put in 10 ml of buffer peptone water. Culture and isolation of *Salmonella* was conducted using International Organization for Standardization (ISO 6579:2002) (ISO, 2002). Typical *Salmonella* isolates were identified sources base on detection of somatic and flagella antigens according to Kauffman-White Scheme (Popoff, 2001).

Antimicrobial resistance testing

Antimicrobial resistance tests were performed by microbroth dilution technique recommended by the US Clinical Laboratory Standard Institute. Resistance testing agents were shown in Table 1. The isolates were considered resistant if the minimum inhibitory concentration was more than or equal to the resistance breakpoint. Quality control of minimum inhibitory concentration was based on standard Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923) (CLSI, 2000).

Data management and statistical analysis

After enter the data, the data were analysed using Stata Version 9.0 (Stata Corporation, College Station, Tx, USA). The proportion of Salmonella positive and 95% of confidence interval (95% CI) were analyzed. Tables were created to display the prevalence of serovars and individual resistance, and frequency of various resistance patterns.

Table 1 List of antimicrobial tested, dilution range and MIC interpretive

Antimicrobials	Dilution range (μg/ml)	Susceptible (S) (μg/ml)	Intermediate (I) (μg/ml)	Resistance (R) ≥ (μg/ml)
Ampicillin (Amp)	1-64	≤ 8	16	32
Ceftriaxone (Cro)	0.25-64	≤ 1	2	64
Chloramphenicol (Chp)	2-64	≤ 8	16	32
Ciprofloxacin (Cip)	0.0156-8	≤ 1	2	4
Gentamicin (Gen)	0.25-16	≤ 4	8	16
Streptomycin (Stm)	32-64	≤ 32	NA	64
Tetracycline (Tet)	4-32	≤ 4	8	16
Trimethoprim-Sulfamethoxazone (Tmp-Smx)	0.12/2.4 - 4/76	≤ 2/38	NA	4/76

Results

Number of *Salmonella* isolates

Of 288 cloacal swabs and 48 environment samples from the farms, there were 37 (12.9%, 95% CI=9.0-16.7%) isolates and 25 (52.1%, 95% CI=37.4-66.7%) *Salmonella* from both groups, respectively. Twenty eight isolates (19.4%, 95% CI=12.9-26.0%) from cloacal swabs and 8 isolates (33.3. 95% CI=13.0-53.6%) from environment were found at the first samplings. Nine farms (37.5%,

95% CI=16.6-58.4%) found *Salmonella* isolates from broilers samples, meanwhile 18 (75%, 95% CI=51.2-90.4%) found *Salmonella* isolates from environment samples.

Prevalence of Salmonella serovars

From Table 2, there were 9 serovars from 62 positive *Salmonella* isolates. *Salmonella* Corvalis (37.8%), *Salmonella* Enteritidis (24.3%) and *Salmonella* Albany (24.3%) were commonly found from the chicken isolates. In contrast, *Salmonella* Virchow was the most frequently observed among isolates from environment (20.0%). *Salmonella* Mbandaka, *Salmonella* Agona, Salmonella Molade and *Salmonella* Weltevreden were found in isolates from environment samples only.

Table 2 *Salmonella* isolated from chickens and bedding samples

Salmonella serovars	Chicken (N=37) n (%)	Environment (N=25) n (%)	Total (N=62) n (%)
S. Corvallis	14 (37.8)	4 (16.0)	18 (29.0)
S. Albany	9 (24.3)	3 (12.0)	12 (19.4)
S. Enteritidis	9 (24.3)	3 (12.0)	12 (19.4)
S. Virchow	4 (10.8)	5 (20.0)	9 (14.5)
S. Senftenberg	1 (2.7)	2 (8.0)	3 (4.8)
S. Weltevreden	0 (0)	3 (12.0)	3 (4.8)
S. Molade	0 (0)	2 (8.0)	2 (3.2)
S. Mbandaka	0 (0)	2 (8.0)	2 (3.2)
S. Agona	0 (0)	1 (4.0)	1 (1.6)

Prevalence of antimicrobial resistant and multiple drug resistant Salmonella

From Table 3, the isolates from both groups were most often resistant to ampicillin. The prevalence of resistance for chloramphenicol, gentamicin, tetracycline, streptomycin and trimethoprim-sulfamethoxazone were low. Table 4 shows that the halves of isolates from broilers were resistant to at least one agent tested. In contrast, more than the halves of isolates from environment samples were susceptible to all antimicrobial agents tested. Only 4 isolates from broiler showed the multiple drug resistant. The highest level of multi-drug resistant of 4 combination agents was found in *Salmonella* from environment but the prevalence was lower than others. *Salmonella* Albany and *Salmonella* Enteritidis were most often resistant to ampicillin. In contrast, *Salmonella* Virchow was completely susceptible to all antimicrobial agents.

Table 3 Prevalence of antimicrobials resistance *Salmonella* isolated (n(%, 95% CI)) from chickens and environment

Agents	Chickens			Environment		
	1st Sampling (N=28)	2nd sampling (N=9)	Total (N=37)	1st sampling (N=8)	2nd sampling (N=17)	Total (N=25)
Amp	14 (50.0)	3(33.3)	17(45.9)	2 (25.0)	5 (29.4)	7(28.0)
Stm	3 (10.7)	0	3(8.1)	1 (12.5)	0	1(4.0)
Gen	2 (7.1)	0	2(5.4)	1 (12.5)	0	1(4.0)
Tmp-Smx	1 (3.6)	0	1(2.7)	0	1 (5.9)	1(4.0)
Chp	0	0	0	0	1 (5.9)	1(4.0)
Tet	0	0	0	0	1 (5.9)	1(4.0)

* Abbreviation for antimicrobial agents and susceptible see Table

Table 4 Prevalence and pattern of antimicrobial multi-resistant from *Salmonella* isolates

Number of resistant antimicrobials	Patterns	Chickens (n=37)		Environment (n=25)	
		resistance	%	resistance	%
0	Susceptible	19	51.4	18	72
1	Amp	14	37.8	5	20
2	Amp-Stm[1c], Gen-Stm[1c]	2	5.4	0	0
3	Amp-Tmp-Smx[1c]			1	4
	Amp-Gen-Stm[1c,1e]	2	5.4		
4	Amp-Tet-Stm-Chp[1e]	0	0	1	4

* Abbreviation for antimicrobial agents and susceptible see Table 1,

[1c] one isolate from chicken

[1e] one isolate from environment

Discussion

In this study, the majority of *Salmonella* serovars in chicken farms were *Salmonella* Corvallis, *Salmonella* Albany, *Salmonella* Enteritidis and *Salmonella* Virchow. Nevertheless, the prevalence rates of *Salmonella* serovars are difference among study sites. *Salmonella* Virchow was the majority in chicken in Nigeria (Fashae et al., 2010). *Salmonella* Anatum and *Salmonella* Typhimurium were the most common in Cambodia (Lay et al., 2010). In 2003-2005, *Salmonella* Enteritidis, *Salmonella* Amsterdam and *Salmonella* Corvallis were the top three serovars in chicken farm in central part of Thailand (Patthanasophon, 2007). Meanwhile *Salmonella* Enteritidis was not found in Nigerian chicken (Fashae et al., 2010). The difference may be explained by different in production system, feed and climate. *Salmonella* Weltevreden, *Salmonella* Molade, *Salmonella* Mbandaka and *Salmonella* Agona was only found in environment. This indicted the bedding may be the source of many *Salmonella* serovars in chicken.

Gastroenteritis *Salmonella* serovars was found in this study. *Salmonella* Enteritidis was associated in national outbreak salmonellaosis in England (Janmohamed et al., 2011). Gastroenteritis cause by *Salmonella* Virchow was reported in Switzerland (Bonalli et al., 2011). Antimicrobial resistance is common among study *Salmonella* serovars from both chickens and environment. The prevalence and the level of multiple drug resistance are more serious among the *Salmonella* isolates from the chickens than those from the environment. *Salmonella* serovars in this study exhibited the highest resistance to ampicillin and completely susceptible to ciprofloxacin. This results are not different from others previous studies (Kilonzo et al., 2008; Cheong et al., 2007).

Ceftriaxone and ciprofloxacin classified as critically important for human medicine (WHO, 2005) were rare in our *Salmonella* isolated. However, the high prevalence of resistance to ampicillin, which is important agents for public health control of infectious diseases, are worrisome. Multi-drug resistance was found in *Salmonella* isolates from chicken and environment, but the prevalence rate in chicken was more than in environment isolates. The evidence of *Salmonella* isolates resistance to multiple drugs in this study is not different from the previous study (Kang et al., 2009).

Salmonella Enteritidis, *Salmonella* Albany and *Salmonella* Corvallis were exhibited the common resistance to ampicillin and rare in gentamicin, streptomycin and trimethoprim-sulfamethoxazone. *Salmonella* Enteritidis in our study was showed the resistance to ampicillin more than the isolates in Korea (Kang et al., 2009). Resistance gastroenteritis *Salmonella* was observed in chicken farms that indicated the resistances become a global public problem. The surveillance of resistance *Salmonella* in food chain and resistance gene would need for further study.

This study indicated resistance contamination is the problem in the chicken farms. Regulation of antimicrobial use should be implemented by the authorities. Also, the surveillance system of antimicrobial use should be done in Thailand.

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ความชุกและการต้านทานต่อยาต้านจุลชีพของเชื้อซัลโมเนลล่าจากฟาร์มไก่ ในจังหวัดเชียงใหม่ และลำพูน ภาคเหนือของประเทศไทย

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บทคัดย่อ การศึกษานี้มีวัตถุประสงค์เพื่อหาความชุกของซีโรวาร์ และการต้านทานต่อยาต้านจุลชีพของเชื้อซัลโมเนลล่าที่เพาะแยกได้จากตัวไก่และสิ่งแวดล้อม จากฟาร์มไก่จำนวน 24 ฟาร์มในจังหวัดเชียงใหม่ และลำพูน ในประเทศไทย จากตัวอย่างไม้ป้ายทวารจากตัวไก่ทั้งหมด 288 ตัวอย่าง และ ตัวอย่างจากสิ่งแวดล้อม จำนวน 48 ตัวอย่าง พบเชื้อซัลโมเนลล่า จำนวน 37 (12.9%, 95%CI=9.0-16.7%) และ 25 (52.1%, 95%CI=37.4-66.7%) ตัวอย่าง ตามลำดับ โดยมาจากการเก็บตัวอย่างครั้งที่ 1 จำนวน 28 (19.4%, 95% CI=12.9-26.0% และ 8 (33.3, 95%CI=13.0-53.6%) ตัวอย่าง ตามลำดับ ชนิดของซีโรวาร์ที่พบมากที่สุดในไก่คือ Salmonella Corvalis, Salmonella Albany, Salmonella Enteritidis, and Salmonella Virchow ซึ่งแตกต่างจากเชื้อที่พบในสิ่งแวดล้อม ที่พบ Salmonella Virchow มากที่สุด การดื้อต่อยาต้านจุลชีพของเชื้อซัลโมเนลล่า พบว่าเชื้อซัลโมเนลล่าจากตัวอย่างทั้ง 2 กลุ่ม ดื้อต่อยาต้านจุลชีพชนิด ampicillin มากที่สุด ซึ่งเป็นรูปแบบของการดื้อต่อยาต้านจุลชีพที่พบมากที่สุดเช่นกัน และพบการดื้อต่อยาหลายชนิดร่วมกัน โดยดื้อต่อยาต้านจุลชีพชนิด ampicillin, tetracycline, streptomycin และ chloramphenicol ร่วมกัน แต่พบความชุกแค่ 4% การศึกษาแสดงให้เห็นว่า การปนเปื้อนเชื้อซัลโมเนลล่าที่ต้านทานต่อยาต้านจุลชีพยังเป็นปัญหาในฟาร์มไก่ ซึ่งการควบคุมการใช้ยาต้านจุลชีพควรจะมีการดำเนินการจากหน่วยงานที่เกี่ยวข้อง และควรจะมีการสร้างระบบการเฝ้าระวังการใช้ยาต้านจุลชีพในประเทศไทย

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