Comparison of prevalence, phenotype, and antimicrobial resistance of *Salmonella* serovars isolated from turkeys in Taiwan

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ABSTRACT *Salmonella* spp. is a foodborne pathogen that causes zoonotic disease worldwide. The aim of this study was to investigate the prevalence of antimicrobial resistance of *Salmonella* isolated from turkey farms in Taiwan. During the past 2 yr, 243 strains of *Salmonella* were isolated from 2,040 samples (11.9%) from turkey farms, including 32.5% (52/160) from the intestines of 12-day-old turkey poults, 14.2% (119/840) from feces collected from the turkey growing periods, and 6.9% (72/1,040) from finishing periods. *S.* Albany (35.0%, 85/243), *S.* Schwarzengrund (23.0%, 56/243), and *S.* Hadar (19.3%, 47/243) were the most common serovars on turkey farms. For these strains, a high frequency of resistance was observed against florfenicol (97.5%), oxytetracycline (89.3%), doxycycline (78.6%), colistin (77.8%), ampicillin (75.7%), amoxicillin (75.3%), trimethoprim-sulfamethoxazole (73.7%), chloramphenicol (69.1%), and nalidixic acid (67.9%). *floR* (63.8%), *tet* (A) (60.5%), bla_{PSE} (57.6%), bla_{TEM} (42.0%), $bla_{\text{CTX-M}}$ (34.2%), *cmlA* (34.2%), and *tet* (D) (29.2%) were the most common resistance genes found in this study. The *int1* gene was identified in 72.4% (176/243) of *Salmonella* isolates in which the conserved region 3' of class 1 integrons also was amplified, whereas none had the *int2* gene. This study demonstrates that imported and fattening turkeys could be a reservoir for *Salmonella* isolates resistant to multiple antimicrobials. These results also reinforce the need to develop strategies and implement specific control procedures to reduce the development of antimicrobial resistance.

Key words: *Salmonella*, zoonosis, food chain, antimicrobial resistance, integrase

2017 Poultry Science 0:1–10
http://dx.doi.org/10.3382/ps/pex293

INTRODUCTION

Salmonella spp. is distributed worldwide and can cause salmonellosis in both animals and humans. In humans, *Salmonella* spp. is one of the most common pathogens causing bacterial foodborne diseases, while in animals, salmonellosis is a common zoonotic disease worldwide. In recent years, *S.* Heidelberg isolated from turkey products caused nationwide outbreaks in the United States, resulting in 78 cases of infection and a recall of up to 3.8 million pounds of turkey products (CDC, 2011). In turkey, *S.* Pullorum, *S.* Gallinarum, and *S.* Arizonae are more pathogenic, while infections caused by other *Salmonella* serotypes are collectively referred to as paratyphoid infections (Barrow, 2000; Quinn et al., 2011). In poults that have paratyphoid infections, clinical signs such as diarrhea, enteritis, and septicemia can be observed, whereas in adult turkeys, paratyphoid infections often lead to subclinical symptoms (Quinn et al., 2011). The common serotypes of turkey origin causing human infections are usually paratyphoid *Salmonella*, including *S.* Heidelberg, *S.* Hadar, and *S.* Stanley (Hafez and Jodas, 2000; Saif et al., 2008; CDC, 2011).

A variety of antimicrobials are used in poultry for disease control, prophylaxis, and growth promotion (Stavric and D'Aoust, 1993; Khachatourians, 1998). Indiscriminate use of antimicrobials in food animals has resulted in the emergence of multiple antibiotic-resistant strains of foodborne pathogens, such as *Salmonella*, *Escherichia coli*, and *Campylobacter*, as well as bacteria endogenous to the microfloras of animals (Davis et al., 1999; van den Bogaard and Stobberingh, 1999). In particular, *Salmonella* isolates from turkeys have been associated with high levels of antimicrobial resistance or multidrug resistance, with studies indicating resistance is even more frequent in *Salmonella* isolates from turkeys than in other livestock species (Schroeter et al., 1998; Zhao et al., 2007, Poppe et al., 2005).

Salmonella spp. acquire antibiotic resistance by random chromosomal mutations, mutation of existing genes, and through mobile genetic elements, such as plasmids, transposons, and gene cassettes in integrons, facilitating the acquisition and dissemination of resistance genes (Okolo, 1986; Recchia and Hall, 1995;

Received June 1, 2017.
Accepted September 14, 2017.

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Fluit and Shmitz, 1999). The resistance gene cassettes carried by integrons can be transferred horizontally to other bacteria through conjugative plasmids and transposons (Shahada et al., 2006). Class 1 integrons are the most frequently detected integron class in *Salmonella* and other Gram-negative organisms (Guerra et al., 2000; White, McIver and Rawlinson, 2001).

The most common serotype of turkey *Salmonella* isolated in the United States in 2010 was *S.* Hadar 19.9% (30/151), followed by *S.* Saintpaul 13.9% (21/151), *S.* Heidelberg 9.3% (14/151), *S.* Schwarzengrund 7.3% (11/151), and *S.* III 18: z4, z23 7.3% (11/151) (USDA, 2012). In addition, *S.* Hadar and *S.* Heidelberg isolates from slaughterhouses in the United States were resistant to multiple antimicrobials, with higher levels of resistance to ampicillin, streptomycin, gentamicin, tetracycline, sulfamethoxazole, and other antimicrobials (Logue et al., 2003; Nayak et al., 2004; Fakhr et al., 2006; FDA, 2013). In Canada, the most common serovar found in turkey was *S.* Heidelberg followed by *S.* Hadar, with multidrug resistance to β-lactams and tetracyclines, including ampicillin, amoxicillin-clavulanic acid, ceftiofur, cefoxitin, and tetracycline; the relevant resistance genes include $bla_{\text{CMY-2}}$, bla_{TEM}, and *tet* (A) (Aslam et al., 2012).

In Taiwan, turkey rearing is not a major poultry industry, and hatching eggs of turkeys are all imported from the United States, with annual production of turkeys between 300,000 and 400,000 birds (COA, 2015). There is a paucity of data concerning the antimicrobial susceptibility status of *Salmonella* isolated from conventional turkey production. Thus, the present study was conducted to investigate the prevalence of *Salmonella*, the serotypes involved, and the antimicrobial susceptibility patterns of *Salmonell*a isolates at different stages from conventional turkey farms in Taiwan.

MATERIALS AND METHODS

Samples Collection

In this study, a total of 2,040 samples from 3 stages of turkey rearing was collected at 20 different farms from March 2014 to February 2016. The 3 stages are 12-day-old turkey poults, growing periods (10 wk), and finishing periods (20 wk). From each turkey-rearing farm, the intestines of 8 turkey poults at the age of 12 d, fresh fecal samples from 42 growing period turkeys, and 52 finisher turkeys were collected. All collected samples were stored on ice and transported to the laboratory within less than 2 h of collection for immediate processing.

Bacterial Isolation and Identification

Salmonella Typhimurium ATCC 14,028 was used as the quality control strain. The isolation method was done according to ISO method 6579/2002/Amd 1:2007 (ISO, 2007). This method was based on the pre-enrichment method in which 25 grams of intestine of poults or turkey fecal sample were taken and mixed with 225 mL of buffered peptone water (BPW; Difco, Detroit, Michigan, USA) at 37°C for 18 ± 2 hours. After overnight incubation, 0.1 mL of the incubated pre-enrichment was transferred to 10 mL of Rappaport-Vassilliadis (RV; Difco, USA) enrichment broth and incubated at 42°C for 24 hours. After incubation, one loop of each selective enrichment broth was streaked onto xylose-lysine-deoxycholate agar (XLD; Oxoid, Basingstoke, Hampshire, UK) at 37°C for 24 hours. Suspected colonies were confirmed through the molecular polymerase chain reaction (**PCR**) method described below. A single colony of each bacterial isolate was suspended in 50 μL of 25 mM NaOH and boiled for 20 minutes. After adding 4 μL of 1 M Tris-HCl (pH 8.0), the suspension was centrifuged, and the supernatant was used as template DNA. The primers were designed to amplify a 244 bp DNA fragment (Chiu and Ou, 1996). The PCR mix was prepared with 3 μL of DNA and 25 μL of 0.4 μM each primer and 5X Fast-Run$^{\text{TM}}$ *Taq* Master Mix (Protech, Taipei, Taiwan). Amplification was performed with the PCR cycling conditions (Chiu and Ou, 1996). The PCR products were then separated using conventional electrophoresis in a 2% agarose gel (Invitrogen, Carlsbad, CA) and ethidium bromide staining.

Pulsed-field Gel Electrophoresis Genotyping

Pulsed-field gel electrophoresis (**PFGE**) was performed according to the protocol developed by the Centers for Disease Control and Prevention (Swaminathan et al., 2001). Restriction digestion was carried out by incubating agarose plug slices with *Xba*I enzyme (Promega, Madison, Wisconsin, USA). PFGE was conducted using a CHEF-Mapper (Bio-Rad Laboratories, Hercules, California, USA) for 18 hours at 6 V/cm at 14°C with an initial switch time of 2.16 seconds and final switch time of 63.8 seconds. *S.* Braenderup H9812 was used as the size standard. The gels were stained with ethidium bromide (Promega, USA) and photographed. The DNA restriction patterns and cluster analysis were performed using GelCompar II V.3.5 (Applied Math, Sint-Martens-Latem, Belgium) and the unweighted pair-group method with arithmetic averages (**UPGMA**). The Dice correlation coefficient was used with a tolerance of 1% to identify similarities between band patterns.

Antimicrobial Susceptibility Testing

Bacterial susceptibility to antimicrobials was tested quantitatively by broth micro-dilution with cation-adjusted Mueller-Hinton broth (Difco, USA) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2007). Sixteen antimicrobials

Table 1. Serotype distribution of *Salmonella* isolates from turkeys in Taiwan, 2014–2016.[1,2,3]

	12-day-old poults ($n = 52$)			Growing (10 wk) ($n = 119$)			Finisher (20 wk) ($n = 72$)		
	Serotypes	Isolates	Percentage	Serotypes	Isolates	Percentage	Serotypes	Isolates	Percentage
1	*S.* Hardar	21	40.3%	*S.* Albany	39	32.8%	*S.* Albany	39	54.2%
2	*S.* Typhimurium	13	25.0%	*S.* Schwarzengrund	37	31.1%	*S.* Schwarzengrund	19	26.4%
3	*S.* Livingstone var. 14+	10	19.3%	*S.* Typhimurium	21	17.6%	*S.* Hadar	8	11.1%
4	*S.* Albany	7	13.5%	*S.* Hadar	18	15.1%	*S.* Stanley	3	4.2%
5	*S.* Tennessee	1	1.9%	*S.* Weltevreden	2	1.7%	*S.* Newport	2	2.8%
6				*S.* Livingstone var. 14+	1	0.8%	*S.* Typhimurium	1	1.4%
7				*S.* Newport	1	0.8%			

[1]The 3 stages are 12-day-old turkey poults, growing periods (10 wk), and finishing periods (20 wk). From each turkey-rearing farm, the intestines of 8 turkey poults at the age of 12 d and fresh fecal samples from 42 growing-period turkeys and 52 finisher turkeys were collected.
[2]The isolation method was done according to ISO method 6579/2002/Amd 1:2007.
[3] The isolation rates of *Salmonella* in the collected samples were as follows: 32.5% (52/160) for intestine from 12-day-old turkey poults, 14.2% (119/840) for feces from grower turkeys, and 6.9% (72/1040) for feces from finisher turkeys.

(7 classes) were used: β-lactams, phenicols, tetracyclines, quinolones, aminoglycosides, polymyxins, and antifolates (Table 1). The criterion of choice was based on antimicrobials for veterinary and human use as directed by the World Health Organization (**WHO**), and the criteria for defining multidrug resistance (**MDR**), extensive drug resistance (**XDR**), and pan-drug resistance (**PDR**) in *Enterobacteriaceae* were according to Magiorakos et al. (2012). The minimum inhibitory concentrations (**MIC**) were determined after 18 h of incubation at 37°C. *Escherichia coli* ATCC 25,922, *Staphylococcus aureus* ATCC 29,213, and *Enterococcus faecalis* ATCC 29,212 were used as antimicrobial susceptibility testing controls in accordance with the CLSI recommendations. The resistance cut-offs that were used were those defined by the CLSI (2008).

Detection of Resistance Determinants

The extracted DNA of *Salmonella* isolates was further examined for detection of resistance genes. PCR was used to determine the presence of select antimicrobial resistance genes, including bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{PSE}, *ampC*, *cmlA*, *floR*, *tet*(A), *tet*(B) *tet*(C), *tet*(D), and *tet*(M) (Mendez et al., 1980; Marshall et al., 1983; Warsa et al., 1996; Koeck et al., 1997; Sandvang et al., 1997; Ng et al., 1999; Pérez-Pérez et al., 2002; Cabrera et al., 2004; Weill et al., 2004). All isolates were screened for the presence of class 1 and 2 integrons by PCR and sequenced to identify gene cassettes. Primers described by Lévesque et al. (1995) and White, McIver and Rawlinson (2001) were used to amplify the variable region of class 1 and 2 integrons, respectively. PCR amplifications were prepared by combining 3 μL of boiled DNA template, with 0.2 μM of each primer, 200 μM dNTP (Protech, Taiwan), 10X reaction buffer (Protech, Taiwan), 1 Unit Pro *Taq* Plus DNA Polymerase (Protech, Taiwan), and sterile water. Amplification was performed with PCR conditions described previously (Lévesque et al., 1995; White, McIver and Rawlinson, 2001). PCR results were analyzed on 2% agarose (Invitrogen, Carlsbad, CA). Amplified PCR products were purified with a QIAquick PCR purification Kit (Qiagen, Valencia, CA) and sequenced using ABI *3730xl* capillary sequencers (Applied Biosystems, Foster City, CA). The DNA sequences were compared using the BLAST online search engine from GenBank at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/blast).

Statistical Analysis

Each experiment was repeated 3 times. The MIC results of the strains were converted to the base 2 logarithm ($\log_2$), and one-way ANOVA followed by Duncan's multiple rang tests analysis were then used to compare whether there were differences between the results of different strains. The chi-square test was used to compare the antimicrobial resistance genes and the serotypes. A P-value < 0.05 was considered statistically significant. All of the analyses were performed using the Statistical Package for the Social Sciences (**SPSS**) V.15.0.

RESULTS

Bacterial Isolation, Identification, and Serovars

From March 2014 to January 2016, turkeys were sampled from 20 turkey farms in Taiwan. The isolation rates of *Salmonella* in the collected samples were as follows: 32.5% (52/160) for intestines from 12-day-old turkey poults, 14.2% (119/840) for feces from grower turkeys, and 6.9% (72/1040) for feces from finisher turkeys (Table 1).

Nine different serotypes of *Salmonella* spp. were detected in this study (Table 1). Of the 243 strains, 52 *Salmonella* strains were isolated from intestines of turkey poults, and their serotypes were *S.* Hadar ($n = 21$), *S.* Typhimurium ($n = 13$), *S.* Livingstone var. 14 + ($n = 10$), *S.* Albany ($n = 7$), and *S.* Tennessee ($n = 1$). In the flocks of grower turkeys, 119 strains of *Salmonella* were isolated, and their serotypes were *S.* Albany ($n = 39$), *S.* Schwarzengrund

(n = 37), *S.* Typhimurium (n = 21), *S.* Hadar (n = 18), *S.* Weltevreden (n = 2), *S.* Livingstone var. 14 + (n = 1), and S. Newport (n = 1). The common serotypes of the 72 *Salmonella* strains isolated from the finishing periods were *S.* Albany (n = 39), *S.* Schwarzengrund (n = 19), and *S.* Hadar (n = 8); other serotypes are shown in Table 1. Interestingly, only 3 serotypes (*S.* Hadar, *S.* Typhimurium, and *S.* Albany) were isolated in whole periods. The predominant serotype identified in poults was *S.* Hadar (40.3%), but *S.* Albany (32.8 and 54.2%) and *S.* Schwarzengrund (31.1 and 26.4%) were the first 2 predominant serotypes in growing and finisher turkeys. *S.* Tennessee, *S.* Weltevreden, and *S.* Stanely were found only in poults, growing, and finisher, respectively.

Antimicrobial Susceptibility Testing

Seven different classes of antimicrobial agents with a total of 16 antimicrobial agents were tested in this study (Table 2). The percentages of resistance, MIC_{50}, and MIC_{90} of the antimicrobial agents observed among isolates in different stages at the turkey farms are shown in Table 2. In these *Salmonella* isolates, high ratios of antimicrobial resistance were detected against florfenicol (97.5%), oxytetracycline (89.3%), doxycycline (78.6%), colistin (77.8%), ampicillin (75.7%), amoxicillin (75.3%), trimethoprim-sulfamethoxazole (73.7%), chloramphenicol (69.1%), and nalidixic acid (67.9%). Medium-levels of resistance to streptomycin (53.1%), flumequine (49.0%), cefazolin (37.4%), amoxicillin-clavulanate (32.5%), and gentamicin (30.9%) also were found in this study. The lowest levels of resistance were enrofloxacin (9.1%) and ciprofloxacin (0.8%).

The MIC levels of *Salmonella* isolates from grower turkeys were higher than those from the turkey poults in all of the tested antimicrobial agents, except for trimethoprim-sulfamethoxazole ($P < 0.05$). In addition, the MIC levels of *Salmonella* isolates from finisher turkeys were higher than those from the turkey poults in all of the tested antimicrobial agents, except for amoxicillin-clavulanate, cefazolin, and streptomycin ($P < 0.05$). For the penicillins, resistance to amoxicillin was significantly higher in the isolates from growers and finishers (81.5 and 80.6%) than in those from poults, whereas the clavulanate combination of amoxicillin substantially decreased the resistance occurrence of these pathogens (34.5 and 34.7%) ($P < 0.05$). In the quinolones, resistance to nalidixic acid and flumequine was also higher in growers and finishers (growers: 84.0 and 53.8%; finishers: 77.8 and 63.9%) than in that from poults ($P < 0.01$). The florfenicol resistance was higher than chloramphenicol in all stages ($P < 0.05$).

In this study, the *Salmonella* isolates also were categorized based on serotypes, and their percentages of antimicrobial resistance are summarized in Table 3 accordingly. Overall, 96.7% (235/243) of the *Salmonella* isolates were resistant to more than 3 antimicrobial agents, with the common serotypes *S.* Schwarzengrund (96.4%; 54/56), *S.* Typhimurium (62.9%; 22/35), and *S.* Albany (75.3%; 64/85) showing resistance to 6 or more antimicrobials (Table 4). Except for *S.* Schwarzengrund, *S.* Typhimurium, and *S.* Albany, the other serotypes in this study were susceptible for enrofloxacin and ciprofloxacin (Table 3). Aminoglycoside resistance in *S.* Schwarzengrund (STR: 94.6% and GEN: 87.5%) and *S.* Typhimurium (STR: 91.4% and GEN: 57.1%) were much higher than the other 2 serotypes [*S.* Albany

Table 2. Antimicrobial susceptibility profiles of *Salmonella* isolates from turkeys in Taiwan (from different source).

Antimicrobial	Breakpoint[1]	12-day-old poults (n = 52)			Growing (10 wk) (n = 119)			Finisher (20 wk) (n = 72)			Total (n = 243)
	(μg/mL)	MIC_{50}	MIC_{90}	R^4 (%)	MIC_{50}	MIC_{90}	R^4 (%)	MIC_{50}	MIC_{90}	R^4 (%)	R^4 (%)
Ampicillin	32	512	1024	46.2[a,c]	1024	1024	82.4[a]	1024	1024	81.9[c]	75.7
Amoxicillin	32	64	1024	48.1[a,c]	1024	1024	81.5[a]	1024	1024	80.6[c]	75.3
Amoxicillin-clavulanate	32	1	16	21.2[a]	16	32	34.5[a]	16	32	34.7	32.5
Cefazolin	32	4	64	21.2[a]	4	32	10.1[a]	4	8	30.6	37.4
Doxycycline	16	16	32	78.8[a,c]	16	64	77.3[a]	32	32	81.9[c]	78.6
Oxytetracycline	16	256	512	80.7[a,c]	512	1024	89.9[a,b]	512	512	94.4[b,c]	89.3
Gentamicin	16	0.5	2	11.5[a,c]	1	64	38.7[a]	0.5	64	31.9[c]	30.9
Streptomycin	64[2]	64	512	48.1[a]	64	1024	55.5[a]	64	512	54.2[b]	53.1
Trimethoprim-sulfamethoxazole	4/76[3]	16	1024	53.8[c]	1024	1024	79.8[b]	1024	1024	86.1[b,c]	73.7
Nalidixic acid	32	8	1024	25.0[a,c]	512	1024	84.0[a,b]	1024	1024	77.8[b,c]	67.9
Flumequine	8	1	32	17.3[a,c]	4	32	53.8[a,b]	16	64	63.9[b,c]	49.0
Enrofloxacin	2	0.25	1	5.8[a,c]	0.5	2	14.3[a]	0.5	1	2.8[c]	9.1
Ciprofloxacin	1	0.125	0.25	0.0[a,c]	0.25	0.5	0.0[a]	0.25	0.5	2.8[c]	0.8
Chloramphenicol	32	8	512	36.5[a,c]	128	512	74.8[a,b]	256	512	81.9[b,c]	69.1
Florfenicol	8	8	512	96.2[a,c]	128	1024	98.3[a]	128	512	97.2[c]	97.5
Colistin	4	4	16	75.0[a,c]	8	16	82.4[a,b]	8	16	73.6[b,c]	77.8

[a]The results of MICs between poults and growing showed significant difference using the analysis of one-way ANOVA ($P < 0.05$).
[b]The results of MICs between growing and finisher showed significant difference using the analysis of one-way ANOVA ($P < 0.05$).
[c]The results of MICs between poult and finisher showed significant difference using the analysis of one-way ANOVA ($P < 0.05$).
[1]Reference from CLSI and BSAC.
[2]Reference from NARMS.
[3]Breakpoint concentration of trimethoprim/breakpoint of sulfamethoxazole.
[4]Resistance percentage.

Table 3. Percentage of antimicrobial resistance among different serotypes.[1]

Antimicrobial agents[a]	*S.* Schwarzengrund ($n = 56$)	*S.* Typhimurium ($n = 35$)	*S.* Stanley ($n = 3$)	*S.* Livingstone var. 14 $^{+}$ ($n = 11$)	*S.* Tennessee ($n = 1$)	*S.* Albany ($n = 85$)	*S.* Hadar ($n = 47$)	*S.* Newport ($n = 3$)	*S.* Weltevreden ($n = 2$)
AMP	91.1	94.3	100	54.5	100	95.3	8.5	100	100
AMO	91.1	94.3	100	54.5	0	94.1	10.6	100	100
AMC	14.3	34.3	66.7	0	0	63.5	2.1	66.7	0
CEF	23.2	82.9	66.7	9.1	100	50.6	0	66.7	0
DOX	83.9	77.1	100	63.6	0	65.9	97.7	100	100
OTC	96.4	80	100	63.6	0	87.1	97.9	100	100
STR	94.6	91.4	100	45.5	0	28.2	19.1	100	0
GEN	87.5	57.1	33.3	0	0	5.9	0	0	0
SXT	100	82.9	100	63.6	100	90.6	2.1	100	100
NAL	96.4	57.1	33.3	18.2	0	94.1	8.5	100	50
UB	58.9	34.3	0	0	0	77.6	6.4	100	100
ENR	35.7	28.6	0	0	0	11.8	0	0	0
CIP	1.8	2.9	0	0	0	0	0	0	0
CHL	98.2	68.6	100	63.6	0	87.1	4.3	100	0
FFC	100	100	100	100	100	97.6	91.5	100	100
COL	75	28.6	33.3	0	0	51.8	76.6	66.7	50

[a]AMP, Ampicillin; AMO, Amoxicillin; AMC, Amoxicillin-clavulanate; CEF, Cefazolin; DOX, Doxycycline; OTC, Oxytetracycline; STR, Streptomycin; GEN, Gentamicin; SXT, Trimethoprim-sulfamethoxazole; NAL, Nalidixic acid; UB, Flumequine; ENR, Enrofloxacin; CIP, Ciprofloxacin; CHL, Chloramphenicol; FFC, Florfenicol; COL, Colistin.

[1]There are statistically significant differences in resistance levels between *S.* Hadar and other *Salmonella* serotypes (*S.* Schwazengrund, *S.* Typhimurium, and *S.* Albany) using the analysis of one-way ANOVA ($P < 0.01$).

Table 4. Distribution of resistance to antimicrobial classes among different serotypes.[1,2]

Serovar[a]	Isolates	Antimicrobial agents classes[b]							
		0	1	2	3	4	5	6	7
Group O:4 (B)									
S. Schwarzengrund	56					1	1	11	43
S. Typhimurium	35					1	12	12	10
S. Stanley	3							2	1
Group O:7 (C1)									
S. Livingstone var. 14 $^{+}$	11	1	2	1		1	2	3	1
S. Tennessee	1					1			
Group O:8 (C2-C3)									
S. Albany	85		1	2	1	6	11	49	15
S. Hadar	47	1			32	10	2	2	
S. Newport	3							1	2
Group O:3, 10 (E1)									
S. Weltevreden	2						1	1	
Total	243	2	3	3	33	20	29	81	72

[a]243 strains of *Salmonella enterica* subspecies *enterica*, belonging to 4 different serogroups (9 different serotypes), were detected in this study.

[b]7 classes of antimicrobial agents as follows: β-lactams, tetracyclines, aminoglycosides, trimethoprim-sulfamethoxazole, quinolones, phenicols and polymyxin.

[1]Criteria for defining MDR, XDR, and PDR in *Enterobacteriaceae*. MDR: non-susceptible to $\geq$1 agent in $\geq$3 antimicrobial categories.

XDR: non-susceptible to $\geq$1 agent in all but $\leq$2 categories. PDR: non-susceptible to all antimicrobial agents listed.

[2]The occurrence of a simultaneous resistance to various antimicrobial agents for *Salmonella* spp. was frequent, illustrating a frequency of more than 80% of resistance to 4 classes of key antimicrobial agents tested, particularly to *S.* Schwarzengrund, *S.* Typhimurium, *S.* Stanely, *S.* Newport. and *S.* Weltevreden.

(STR: 28.2% and GEN: 5.9%) and *S.* Hadar (STR: 19.1% and GEN: 0.0%)] ($P < 0.05$). From the phenicols groups, florfenicol resistance in *S.* Hadar was extensively higher than chloramphenicol ($P < 0.01$). The occurrence of a simultaneous resistance to various antimicrobial agents for *Salmonella* spp. was frequent, illustrating a frequency higher than 80% of resistance to 4 classes of key antimicrobial agnets tested, particularly to *S.* Schwarzengrund, *S.* Typhimurium, *S.* Stanely, *S.* Newport, and *S.* Weltevreden (Table 4). However, 2 isolates (*S.* Livingstone var. 14+ and *S.* Hadar) were susceptible for all antimicrobial agents tested in this study. In addition, the resistance levels found between *S.* Hadar and other *Salmonella* serotypes (*S.* Schwarengrund, *S.* Typhimurium, and *S.* Albany) were significantly different, as calculated by one-way ANOVA.

Prevalence of Antimicrobial Resistance Genes

Detected β-lactam resistance genes included $bla_{\text{PSE-1}}$ (140/243), bla_{TEM} (102/243), $bla_{\text{CTX-M}}$ (83/243), and *ampC* (35/243), but other genes including bla_{SHV},

ampC$_{MOXM}$, *ampC*$_{DHAM}$, and *ampC*$_{ACCM}$ were not detected in this study (Table 5). *ampC*$_{EBCM}$ and *ampC*$_{FOXM}$ were detected in *S.* Typhimurium only. Chloramphenicol resistance genes detected in this study were *floR* (155/243) and *cmlA* (83/243) (Table 5). Interestingly, *flo*R was not detected in all *S.* Hadar, and only one isolate was *cml*A positive. Frequently detected tetracycline resistance genes included *tet*(A) (147/243), *tet*(D) (71/243), *tet*(B) (10/243), *tet*(M) (17/243), and *tet*(C)(6/243) (Table 5). In addition, there is a correlation calculated by the chi-square test between *tet*(A) and *tet*(D) in all serotypes, except for *S.* Tennessee, *S.* Hadar, and *S.* Newport. Only one antimicrobial resistance gene (*cml*A) was detected in *S.* Tennessee. There is no correlation between antimicrobial resistance genes and serotypes (*S.* Schwarzengrund, *S.* Typhimurium, *S.* Albany, and *S.* Hadar) based on the analysis using the chi-square test. The *int1* gene, encoding the integrase of class 1 integrons, was identified in 176 isolates (72.4%) in which the conserved region 3' of this type of integron also was amplified. All isolates were negative for the *int2* gene. Table 5 shows various resistance genes found in each of the serovars isolated from turkey farms.

Classification of Class 1 Integron

Class 1 integrons were detected in 174 (72.4%) isolates, of which more than half were found in the following serotypes: *S.* Newport (3/3), *S.* Stanley (3/3), *S.* Tennessee (1/1), *S.* Schwarzengrund (54/56), *S.* Albany (79/85), and *S.* Typhimurium (23/35). Class 2 integrons were not detected in any of the isolates. The *drfA1-orfC/bla*$_{PSE-1}$ gene cassettes arrangement was found in 83 integron-positive *Salmonella* isolates (47.7%), followed by *drfA12-orfF-aadA2* gene cassettes with the percentage at 38.50% (67/174). The *bla*$_{PSE-1}$ gene cassette was 6.90% (12/174), and the *aadA22* gene cassette was 4.60% (8/174). The results of serotypes and their percentages of integrons are summarized in Table 5. From these results, the percentage of integrons in *S.* Hadar was much lower than in the other serotypes (8/47, 17.0%), and all integrons in *S.* Hadar were *bla*$_{PSE-1}$ gene cassette positive. Most of the *drfA1-orfC/bla*$_{PSE-1}$ gene cassettes and *drfA12-orfF-aadA2* gene cassettes were found in integrons of *S.* Albany and *S.* Schwarzengrund, respectively. The serotypes with multidrug resistance, such as *S.* Newport (3/3), *S.* Stanley (3/3), *S.* Schwarzengrund (54/56), and *S.* Albany (79/85), were mostly found to harbor the class 1 integrons.

DISCUSSION

The current outbreak of human *Salmonella* infection associated with turkey meat has highlighted risks associated with the production, handling, and preparation of meat and poultry products (CDC, 2011). Furthermore, multidrug-resistant *Salmonella* spp. has

Table 5. Distribution of antimicrobial resistance genes among different serotypes.[1,2]

Serovar	N	β-lactams								Phenicols		Tetracyclines					Integron	
		Bla TEM	*Bla* PSE	*Bla* CTX-M	*Bla* SHV	*ampC*	*ampC* MOXM	*ampC* CITM	*ampC* DHAM	*ampC* ACCM	*ampC* EBCM	*ampC* FOXM	*cmlA*	*floR*	*tet* (A)	*tet* (B)	*tet* (C)	*tet* (D)	*tet* (M)	*int1*	*int2*
Group O:4 (B)																					
S. Schwarzengrund	56	49	11	11	0	0	0	0	0	0	0	0	55	50	53	1	5	40	0	54	0
S. Typhimurium	35	23	23	30	0	10	0	10	0	0	8	4	10	25	26	8	1	18	6	23	0
S. Stanley	3	3	2	3	0	0	0	0	0	0	0	0	3	0	3	0	0	3	0	3	0
GroupO:7 (C1)																					
S. Livingstone var. 14 $^{+}$	11	8	0	1	0	1	0	0	0	0	0	0	1	6	6	1	0	4	9	5	0
S. Tennessee	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
GroupO:8 (C2-C3)																					
S. Albany	85	14	80	33	0	0	0	0	0	0	0	0	12	72	8	0	0	4	2	79	0
S. Hadar	47	2	21	1	0	0	0	0	0	0	0	0	1	0	47	0	0	0	0	8	0
S. Newport	3	3	3	3	0	1	0	1	0	0	0	0	0	2	2	0	0	0	0	3	0
Group O:3, 10 (E1)																					
S. Weltevreden	2	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0
Total	243	102	140	83	0	12	0	11	0	0	8	4	83	155	147	10	6	71	17	176	0

[1]Based on their mechanism, antimicrobial resistance genes were classified into these groups as follows: Penicillinases (broadspectrum β-lactamases and ESBL): *bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{PSE}$, *bla*$_{SHV}$; Cephalosporinases (AmpC β-lactamases): *ampC*, *ampC*$_{MOXM}$, *ampC*$_{DHAM}$, *ampC*$_{CITM}$, *ampC*$_{ACCM}$, *ampC*$_{EBCM}$, *ampC*$_{FOXM}$; Phenicol efflux pump: *cmlA* (Type E-1) and *floR* (Type E-3); tetracycline efflux pump: *tet*(A), *tet*(B), *tet*(C), *tet*(D); Ribosomal protection resistance gene of tetracycline: *tet*(M); based on the differences in the integrase gene, integrons are classified into several families, including classes 1 and 2.

[2]There is no correlation between antimicrobial resistance genes and serotypes (*S.* Schwarzengrund, *S.* Typhimurium, *S.* Albany, and *S.* Hadar) based on the analysis using the chi-square test.

been frequently detected in humans and animals worldwide (Winokur et al., 2000; Lu et al., 2014; Routh et al., 2015; Sinwat et al., 2016), with many countries already utilizing monitoring programs for antimicrobial resistance in *Salmonella*. Hence, many research reports on this topic are available (USDA, 2000; White et al., 2001; EFSA, 2008). For example, several studies on *Salmonella* in the United Kingdom were conducted at 252 turkey farms (Featherstone et al., 2010), with results showing that the isolation rate at the farm level was 34.1% (86/252) and that the most prevalent serovar were *S.* Kottbus (17.9%), followed by *S.* Typhimurium (6.0%) and *S.* Derby (5.2%; 13/252). Another report from a British turkey breeder farm and hatchery demonstrated that *S.* Kottbus and *S.* Senftenberg were the most frequently isolated (Mueller-Doblies et al., 2013). Aury et al. (2010) conducted an investigation into turkey *Salmonella* in France, and the results showed that the 5 most common serotypes were *S.* Derby (29.2%), *S.* Enteritidis (11.7%), *S.* Typhimurium (10.1%), *S.* Hadar (8.4%), and *S.* Mbandaka (6.8%) (Aury et al., 2010). In Canada, *S.* Heidelberg and *S.* Hadar were the most prevalent serotypes in turkeys (Aslam et al., 2012). A report indicated that the most commonly detected serotypes among turkey isolates in the United States were *S.* Hadar (19.9%), *S.* Saintpaul (13.9%), *S.* Heidelberg (9.3%), *S.* Schwarzengrund (7.3%), and *S.* Albany (4.0%) (USDA, 2012). In the present study, the most common serotypes of the *Salmonella* in turkeys were *S.* Albany (35.0%; 85/243), *S.* Schwarzengrund (23.0%; 56/243), *S.* Harda (19.3%; 47/243), and *S.* Typhimurium (14.4%; 35/243). It can be noted that *S.* Hadar was among the most common serotypes in Taiwan, France, Canada, and the United States, but not in the United Kingdom. *S.* Typhimurium also was frequently isolated in Taiwan, the United Kingdom, and France. The abovementioned results from different countries indicate that geographical factors may have contributed to the varying distribution of prevalence of *Salmonella* serotypes (Lewerin et al., 2011). Thus, each country or geographic region will need to conduct epidemiological surveillance of the *Salmonella* serotypes in a proactive manner.

Vertical transmission of *Salmonella* has been demonstrated experimentally in chickens (Keller et al., 1995). Horizontal transmission in hatcheries has been suggested in some studies as well (Angen et al., 1996; Christensen et al., 1997; Skov et al., 1999). In Taiwan, it is mandatory by law to quarantine imported young turkeys and imported embryonated turkey eggs for 10 d after being imported (BAPHIQ, 2009). Therefore, whether there is vertical transmission or horizontal transmission during the quarantine period merits further studies when the laws and regulations permit.

Salmonella isolated from turkey meat products in the United States was found to be highly resistant to antimicrobials such as ampicillin, tetracycline, and streptomycin (FDA, 2013). In the present study, the results showed that over half of *Salmonella* isolates were resistant to streptomycin (53.1%), nalidixic acid (67.9%), trimethoprim-sulfamethoxazole (73.7%), amoxicillin (75.3%), ampicillin (75.7%), colistin (77.8%), doxycycline (78.6%), oxytetracycline (89.3%), and florfenicol (97.5%) (Table 2), all of which are frequently administered on turkey farms in Taiwan. The results also indicated a similar pattern in high resistance to antimicrobials such as streptomycin, ampicillin, and oxytetracycline in Taiwan. It also can be noted that a high prevalence of ampicillin- and amoxicillin-resistant strains was observed in almost all of the *Salmonella* serotypes in this study, with the exception of *S.* Hadar and *S.* Livingstone var. 14 $^{+}$. In addition, when comparing the MIC levels of the same antimicrobial agent in the *salmonella* isolates from finishing turkeys and those from turkey poults, the MIC levels were found to be significantly higher ($P < 0.05$) in the finishing turkeys than those in the turkey poults for almost all the antimicrobial agents tested in this study, except for amoxicillin-clavulanate, cefazolin, and streptomycin (Table 2). This might reflect the effect of long-term antimicrobial administration in the turkey-rearing industry in Taiwan. During the period of turkey-rearing (approximately 4.5 to 5 mo), antimicrobial agents used in turkeys are often administered in a way that increases antimicrobial resistance, e.g., by subtherapeutic dosage or repeated mass treatments, long-term administration of antimicrobials, or addition to feed and water for prophylactic purposes (Viswanathan, 2014). These practices may have caused the emergence of resistant *Salmonella* strains (EFSA and ECDC, 2014). In Taiwan, chloramphenicol has been prohibited for use in food-producing animals since 2002 (COA, 2002). After chloramphenicol was banned, florfenicol was used to replace chloramphenicol in clinical treatment (Neu and Fu, 1980; White et al., 2000). Some studies have shown that the frequent and intensive administration of antimicrobials may lead to the spread of plasmids, transposons, or integrons carrying relevant genes for antimicrobial resistance among different strains of bacteria (Bennett, 2008; Nikaido, 2009). Since the aforementioned materials could also harbor resistance genes of other classes of antimicrobials, multiple antimicrobial resistances could then ensue (Levy and Marshall, 2004; Salyers et al., 2004). This might explain the ubiquity of antimicrobial resistance found in different serotypes of *Salmonella* on Taiwan's turkey farms. The above points underline the need to address these issues, such as the practice of administering antimicrobials and the emergence of antimicrobial resistance facing the turkey-rearing industry in Taiwan.

In the present study, 72.4% of the *Salmonella* isolates (176/243) were found to carry the *intI1* gene, with no *intI2* gene detected. Of the 176 isolates with the *intI1* gene, different serotypes showed a varying degree of *intI1* gene distribution with the highest proportions in *S.* Stanley 100% (3/3), *S.* Tennessee 100% (1/1), and *S.* Newport 100% (3/3), followed by *S.*Schwarzengrund 96.4% (54/56), *S.* Albany

92.9% (79/85), *S.* Typhimurium 65.7% (23/35), *S.* Livingstone var. 14 + 45.5% (5/11), and *S.* Hadar 17.0% (8/47). According to many literatures, there were 9.1 to 83.6% of *Salmonella* strains carrying class 1 integrons, which are genetic materials that confer multidrug resistance (Leverstein-van Hall et al., 2002; Chen et al., 2004; Dessie et al., 2013; Hsu et al., 2013; Siriken et al., 2015). Thus, it can be deduced that the *int1* gene had a strong presence in most of the turkey *Salmonella* in Taiwan. In addition, the *int1* genes detected in the present study mostly belonged to the *drfA1-orfC*/$bla_{\text{PSE-1}}$ gene cassettes and *drfA12-orfF-aadA2* gene cassettes, and this finding was consistent with the study results by Hsu et al. (2013). Moreover, detection of the antimicrobial resistance genes was performed in this study, and the results showed the top 4 of the most isolated serotypes (*S.* Albany, *S.* Schwarzengrund, *S.* Hadar, and *S.* Typhimurium) all carried multi-antimicrobial resistance genes. A high prevalence of ampicillin- and amoxicillin-resistant strains was observed in this study. In terms of the β-lactam resistance genes, bla_{PSE} and $bla_{\text{CTX-M}}$ were detected in the *S.* Albany isolates at the percentages of 94.1% (80/85) and 38.8% (33/85), respectively; bla_{TEM} was detected in 87.5% (49/56) of the *S.* Schwarzengrund isolates; bla_{PSE} was detected in 44.7% (21/47) of the *S.* Hadar isolates; $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{PSE} were detected in 85.7% (30/35), 65.7% (23/35), and 65.7% (23/35), respectively, of the *S.* Typhimurium isolates. It can be noted that although 44.7% (21/47) of the *S.* Hadar isolates carried the gene of bla_{PSE}, antimicrobial resistance to ampicillin and amoxicillin was found to be relatively low (8.5 and 10.6%, respectively) in the *S.* Hadar isolates. A high prevalence of chloramphenicol and florfenicol resistance strains was observed in this study, indicating that active efflux pumps (*cmlA* and *floR* gene) played an important role in intrinsic and acquired resistance against chloramphenicol and/or florfenicol (Braibant et al., 2005; Chang et al., 2015). In this study, *floR* gene was detected in 84.7% (72/85) of the *S.* Albany isolates, while *cmlA* and *floR* genes were detected in 98.2% (55/56) and 89.3% (50/56), respectively, of the *S.* Schwarzengrund isolates. Amphenicol-related resistance genes were seldom detected in the *S.* Hadar isolates in this study, whereas *floR* gene was detected in 71.4% (25/35) of the *S.* Typhimurium isolates. Again, it can be noted that antimicrobial resistance to florfenicol was fairly high (91.5%) in the *S.* Hadar isolates, despite only one out of the 47 *S.* Hadar isolates being detected with *cmlA* gene. For tetracycline-related resistance genes, only a few were detected in the *S.* Albany isolates, whereas many were detected in the *S.* Schwarzengrund and *S.* Typhimurium isolates with *tet* (A) and *tet* (D) found to be the main resistance genes. *tet* (A) and *tet* (D) were detected in 94.6% (53/56) and 71.4% (40/56) of the *S.* Schwarzengrund isolates; *tet* (A) and *tet* (D) were detected in 74.3% (26/35) and 51.4% (18/35) of the *S.* Typhimurium isolates. For *S.* Hadar, *tet* (A) was detected in all of the *S.* Hadar isolates (47/47). There were some discrepancies between the detection rates of resistance genes and the actual levels of antimicrobial resistance for some *Salmonella* serovars in this study. For instance, 44.7% (21/47) of the *S.* Hadar isolates were found to have bla_{PSE} gene, but only 8.5 and 10.7% of the *S.* Hadar isolates showed resistance to ampicillin and amoxicillin, respectively. The underlying reason for this phenomenon needs to be further investigated. In addition, *S.* Hadar isolates in this study also had high resistance to florfenicol (91.5%), but surprisingly none (0%) of the *S.* Hadar isolates was detected to harbor *floR* gene, and only one of the *S.* Hadar isolates (2.1%) had *cmlA* gene. Taking account of this result and the other finding that only 4.3% of the *S.* Hadar isolates were resistance to chloramphenicol, which belongs to the same class of antimicrobial agent as florfenicol, it might suggest that other mechanisms of antimicrobial resistance could be involved (data not shown), such as over-expression of the multidrug efflux system: *mdfA*, *floR*, *cmlA*, or AcrAB-TolC system (Andersen et al., 2015, Baucheron et al., 2004). This kind of discrepancy also was observed in the *S.* Albany isolates, in which the resistances to doxycyline and oxytetracycline were 65.9 and 87.1%, while the detection rates of *tet* (A) and *tet* (D) genes were only 9.4 and 4.7% each. There might be other mechanisms involved, and further studies and tests are needed for elucidation.

Nontyphoidal *Salmonella* spp. has become increasingly resistant to clinically important antimicrobial agents, and this is causing a public health concern. This is the first paper, to the best of our knowledge, that provides data about the current status of the antimicrobial susceptibility of nontyphoidal *Salmonella* isolated from commercial turkey farms in various regions of Taiwan. Since Taiwan's turkey-rearing industry relies heavily on imported turkey chicks from the United States, further studies are needed to clarify whether young turkeys are already infected with *Salmonella* at hatcheries when being imported. Approaches based on ecology and the results of this study underline the need for more proactive surveillance of antimicrobial resistance and the urgency in improvement of poultry production practices in order to reduce and even eliminate food contamination by multidrug-resistant bacteria. Moreover, an approach based on ecology and epidemiology is needed to fully understand the emergence, transmission, and persistence of antibiotic-resistant *Salmonella.*

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