Antimicrobial Resistance Genes in *Salmonella enterica* Isolates from Poultry and Swine in Thailand

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ABSTRACT. A total of 184 resistant *Salmonella enterica* isolated from poultry and swine were investigated for the distribution of and associations between various antimicrobial resistance genes. All the isolates were screened for the presence of class 2 and 3 integrase genes and 18 resistance genes corresponding to their resistance phenotypes. None of the isolates carried class 2 and 3 integrons. The investigated resistance genes were responsible for resistance in 78% of the isolates. All the strains harboring more than one resistance gene were resistant to three or more antimicrobials. The *bla*_{TEM}, *cmlA*, *tetA*, *dfrA12*, *sul3*, *aadA1* genes were detected in the majority of strains resistant to ampicillin (87%), chloramphenicol (63%), tetracycline (60%), trimethoprim (42%), sulphonamides (42%) and streptomycin/spectinomycin (61%), respectively. Two ciprofloxacin-resistant isolates had a single point mutation leading to Ser-83-Phe in GyrA or Thr-57-Ser in ParC. Statistical analysis revealed good correlation between the presence of antimicrobial resistance genes and corresponding resistance phenotype ($p < 0.01$). The results indicated that the resistance genes play a major role in conferring resistance among the *Salmonella* isolates investigated.

KEY WORDS: antimicrobial resistance, resistance genes, *Salmonella enterica*.

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Food-borne diseases caused by nontyphoid *Salmonella enteric* serovars represent an important public health problem worldwide [24, 30]. In the last two decades, multidrug resistant (MDR) *Salmonella* isolates has been increasing and become a major health hazard [1, 3, 13]. These MDR *Salmonella* strains can be a direct threat to human health when their multidrug resistance phenotype interferes with the efficacy of antimicrobial treatment or an indirect threat when resistance is transferred to other human pathogens. The routine use of antimicrobials in food-producing animals for any purposes, including infection treatment, disease prevention and growth promotion has been a major factor in development and widespread dissemination of antimicrobial resistance in bacteria that are subsequently transferred to humans through the food chain. As a result, the European Union has banned the continuous feeding of antimicrobials used in human medicine to healthy animals for growth promotion purpose and recommended risk-based evaluations for all antimicrobial growth promoters [18].

In Thailand, antimicrobials have been extensively used in food animal production for decades. The country has faced the increasing-public health problem due to the misuse and overuse of these substances in food animals particularly MDR *Salmonella* that has been isolated along the food chain [1, 13, 23]. Use of antimicrobials in food animals has been regulated by the Thai Department of Livestock Development and all antimicrobials intended to use in food animals must be approved by Thai Food and Drug Administration. The relevant authorities in Thailand have attempted to rap-

idly phase out antimicrobials as growth promoters and prohibited all antimicrobials being used as feed additives since 2005. Currently, the new regulatory policy for controlling use of antimicrobial feed additives is in the drafting process. In this case, manufacturers will be required to submit applications for evaluation and authorization of the current-existing and new feed additive antimicrobials for animals as growth promoter. Public health risk from development and dissemination of antimicrobial resistance in foodborne and human pathogens must be assessed and this scientific-based data must be readily available for pre-approval evaluation. Hence, surveillance of antimicrobial resistance in bacteria isolated from food animal reservoirs and study of mechanisms underlying the emergence and dissemination of antimicrobial resistance is crucially needed.

In our previous study, the prevalence, molecular characteristics and transferability of class 1 integrons in *S. enterica* from healthy swine and poultry were investigated [15]. In this study, we further characterized the molecular mechanisms of multidrug resistance among the *Salmonella* isolates. The objectives were to investigate distribution and associations of various resistance genes in MDR *Salmonella* isolates.

MATERIALS AND METHODS

Bacterial strains and antimicrobial susceptibility test: A total of 184 *Salmonella* isolates previously isolated were studied [8, 15]. They were originated from poultry ($n=80$) and swine ($n=104$) and comprised of 34 serotypes including 24 Stanley, 19 Typhimurium, 15 Enteritidis, 13 Rissen, 13 Bsilla, 11 Weltevreden, 10 *Salmonella* subspecies I, 9 of each Anatum, Corvallis and Kedougou, 6 Albany, 5

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Amsterdam, 3 of each Altona, Agona, Orion, Virchow and Worthington, 2 of each Bovismorbifican, Give, Madjorio, Kentucky, Poona and Senftenberg, and another 10 serovars presented by only one isolate i.e. Bareilly, Blockley, Eppendorf, Kingston, Paratyphi B2, Panama, Schwarzengrund, Singapore, Thomson and Virginia. A hundred and forty four strains were obtained from the strain collection at National Institute of Animal Health (NIAH), Department of Livestock, Bangkok during 2003–2005. The other strains were isolated from samples (feces, rectal swabs, drinking water and feed) collected from healthy poultry and swine during 2005–2006. All of the *Salmonella* strains were isolated and tested for their biochemical characteristics using methods described in ISO6579:2002 (E) [14] and serotyped by slide agglutination using the Kauffmann-White serotyping schemes [25]. All the *Salmonella* isolates were subjected to antimicrobial susceptibility testing and the minimum inhibitory concentrations (MICs) were determined using a 2-fold agar dilution technique according to Clinical and Laboratory Standards Institute guidelines (CLSI) formerly NCCLS [20]. The antimicrobial agents tested and the breakpoints for clarifying *Salmonella* as resistant were as follows: ampicillin (32 µg/ml), chloramphenicol (32 µg/ml), ciprofloxacin (4 µg/ml), gentamicin (8 µg/ml), spectinomycin (128 µg/ml), streptomycin (32 µg/ml), sulfamethoxazole (512 µg/ml), tetracycline (16 µg/ml) and trimethoprim (16 µg/ml) [11, 21]. Multidrug resistance was defined as isolates being resistant to 3 or more different classes of antimicrobials [22]. All the antimicrobials were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).

All of the isolates used were previously classified as resistant to at least one antimicrobial agent [15]. Resistance rates to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, spectinomycin, streptomycin, sulfamethoxazole, tetracycline and trimethoprim were 56%, 32%, 2%, 14%, 56%, 69%, 76%, 66% and 38%, respectively. Fifty-three percent of the isolates were resistant to 3 different classes of antimicrobials or more and considered multidrug resistance (MDR). Fifteen percent of the *Salmonella* strains carried class 1 integrons with resistance gene cassettes and 5% of the isolates harbored SGI1-A or -F [15].

Polymerase Chain Reaction (PCR) and DNA sequencing: The 184 resistant isolates were screened for class 3 integrase, *intI3* and 18 resistance genes corresponding to their resistance phenotypes using PCR. Ampicillin-resistant isolates ($n=103$) were screened for the presence of the *bla*_{PSE-1} and *bla*_{TEM} genes. Chloramphenicol-resistant isolates ($n=59$) were investigated for the *catA*, *catB* and *cmlA* genes. Gentamicin-resistant strains ($n=26$) were screened for the *aadB* gene. All strains resistant to tetracycline ($n=86$) were examined for the presence of the *tetA* and *tetB* genes. Trimethoprim-resistant isolates ($n=69$) were investigated for the *dfrA1*, *dfrA10* and *dfrA12* genes. Spectinomycin-resistant isolates ($n=103$) were screened for the presence of the *aadA1* and *aadA2* genes. Strains resistant to streptomycin ($n=127$) were investigated for the *aadA1*, *aadA2*, *strA* and *strB* genes. All the strains resistant to sulfamethoxazole

($n=139$) were screened for the presence of the *sul1*, *sul2* and *sul3* genes.

For all ciprofloxacin-resistance isolates ($n=3$), mutations in the quinolone resistance-determining regions (QRDRs) of the *gyrA*, *gyrB*, *parC* and *parE* genes were determined. The sequences were analyzed using the BLAST search available at the website of the National Center for Biotechnology Information. Two *Salmonella* isolates susceptible to ciprofloxacin were used as control.

Template DNA of all isolates was prepared by the whole cell boiled lysate procedure [16]. PCR was performed in a final volume of 25 µl using PCR master mix of Eppendorf® MasterMix (Eppendorf, Hamburg, Germany) according to the manufacturer's protocol in a PCR Sprint Thermocycler® (Thermo Electron Corporation®, Cambridge, UK). All primers used are listed in Table 1. The following thermal cycles were used to amplify all resistance genes and the QRDRs: one cycle at 94°C for 5 min; 30 cycles of 94°C for 45 s, 57°C for 45 s, and extension at 72°C for 1 s; and a final extension at 72°C for 5 min. The *intI3* gene was amplified using the same denaturation and annealing conditions, except that the extension time was changed to 45 sec. The representatives of PCR products were purified using the QIAQuick Gel Extraction kit (Qiagen, Hilden, Germany) and submitted for sequencing at Macrogen Inc. (Seoul, South Korea). *Salmonella* Paratyphi B var Java [28] and pAV3.5 [29] were used as positive controls for *intI2* and *intI3*, respectively.

Realtime PCR: All the isolates were screened for the presence of class 2 integrase gene, *intI2*, using realtime PCR. PCR reactions were carried out in a final volume of 25 µl using Biotools QuantiMix EASY SYG Kit (Biotools B&M Labs S.A., Madrid, Spain) as described by the manufacturer in a Rotor-Gene™ 3000 Real Time Thermal Cycler (Corbett Research, Sydney, Australia). Amplification conditions were 50°C for 2 min, 95°C for 2 min, 40 cycles of 95°C for 15 s, 60°C for 30 s followed by holding at 4°C. *Salmonella* Paratyphi B var Java was used as positive control for *intI2* [28]. The specificity of realtime PCR was confirmed by melting curve analysis and detection of a 247 bp PCR-amplicon by gel electrophoresis.

Statistical analysis: All statistical analysis was carried out using STATA software Version 8.0 (STATA Corp., College Station, TX, U.S.A.). The significance level of association between having resistance genes and resistance phenotype was determined using Fisher's exact test. Comparison among median MIC level of isolates with different number of resistance gene (s) was performed using Kruskal-Wallis test.

RESULTS

No class 2 or 3 integrons were detected in the *Salmonella* isolates in this study. The distribution of the various resistance genes and the prevalence of the corresponding serovars are shown in Table 2. The investigated resistance genes were responsible for resistance in 78% of the isolates

Table 1. Primers used in this study

Target gene or region	Primer	Sequence of primers (5'–3')	Size (bp)	Reference
<i>aadA1</i>	aadA1-F aadA1-R	CTCCGAGTGGATGGCGG GATCTGCGCGCGAGGCCA	631	[8]
<i>aadA2</i>	aadA2-F aadA2-R	CATTGAGCGCCATCTGGAAT ACATTTGCTCATCGCCGGC	500	[8]
<i>aadB</i>	aadB-F aadB-R	CTAGCTGCGCGAGATGAGC CTCAGCCGCTCTGGGCA	300	This study
<i>bla_{PSE-1}</i>	bla _{PSE-1} -F bla _{PSE-1} -R	GCAAGTAGGGCAGGCAATCA GAGCTAGATAGATGCTCACAA	422	[8]
<i>bla_{TEM}</i>	bla _{TEM} -F bla _{TEM} -R	ATCAGTTGGGTGCACGAGTG ACGCTCACCGGCTCCAGA	608	[8]
<i>catA</i>	catA-F catA-R	CCAGACCGTTCAGCTGGATA CATCAGCACCTTGTGCGCT	454	[8]
<i>catB</i>	catB-F catB-R	CGGATTGAGCCTGACCACC ATACGCGGTACCTTCCTG	461	[8]
<i>cmlA</i>	cmlA-F cmlA-B	TGGACCGCTATCGGACCG CGCAAGACACTTGGGCTGC	641	[7]
<i>dfrA1</i>	dfrA1-F dfrA1-R	CAATGGCTGTTGGTTGGAC CCGGCTCGATGTCTATTGT	254	[8]
<i>dfrA10</i>	dfrA10-F dfrA10-R	TCAAGGCAAATTACCTTGGC ATCTATTGGATCACCTACCC	432	This study
<i>dfrA12</i>	dfrA12-F dfrA12-R	TTCGCAGACTCACTGAGGG CGGTTGAGACAAGCTCGAAT	330	[8]
<i>strA</i>	strA-F strA-R	TGGCAGGAGGAACAGGAGG AGGTCGATCAGACCCGTGC	405	This study
<i>strB</i>	strB-F strB-R	GCGGACACCTTTCCAGCCT TCCGCCATCTGTGCAATGCG	621	This study
<i>sul1</i>	sul1-F sul1-R	CGGACGCGAGGCCTGTATC GGGTGCGGACGTAGTCAGC	591	[6]
<i>sul2</i>	sul2-F sul2-R	GCGCAGGCGCGTAAGCTGAT CGAAGCGCAGCCGCAATTC	514	This study
<i>sul3</i>	sul3-F sul3-R	GGGAGCCGCTTCCAGTAAT TCCGTGACACTGCAATCATTA	500	[7]
<i>tetA</i>	tetA-F tetA-R	GCTGTCGGATCGTTTCGG CATTCCGAGCATGAGTGCC	658	[8]
<i>tetB</i>	tetB-F tetB-R	CTGTGCGGCATCGGTCAT CAGGTAAAGCGATCCCACC	615	[8]
<i>intI2</i>	intI2 intI2	GGCAGACAGTTGCAAGACAA AAGCGATTTCTGCGTGTTC	247	This study
<i>intI3</i>	intI3-F intI3-R	CCGGTTCAGTCTTTCCTCAA GAGGCGTGTACTTGCCATCAT	155	This study
QRDR of <i>gyrA</i>	gyrA-F gyrA-R	GCTGAAGAGCTCCTATCTGG GGTCGGCATGACGTCCGG	436	This study
QRDR of <i>gyrB</i>	gyrB-F gyrB-R	GCGCGCTCGATTTAGCCG TGATAGCGCAGCTTGTCCG	287	This study
QRDR of <i>parC</i>	parC-F parC-R	GTACGTGATCATGGATCGTG TTCCTGCATGGTGCCGTCG	390	This study
QRDR of <i>parE</i>	parE-F parE-R	GCGATCGCGAATATCAGGCG CAGTTGTTCCAGTACGCCC	364	This study

and the existence of each resistance gene did not appear to be specific to a particular serovar. Most of ampicillin-resistant strains (93%) contained either *bla_{PSE-1}* and/or *bla_{TEM}*, of which the *bla_{TEM}* gene was the most commonly-identified genes (87%). The majority of chloramphenicol- (63%) and gentamicin- (88%) resistant isolates carried *cmlA* and *aadB* genes respectively. The *tetA* gene was detected in the

majority of tetracycline-resistant strains (60%) and the *dfrA12* gene was most prevalent among trimethoprim-resistant strains (42%). Most of sulphonamide-resistant strains (76%) contained at least one *sul* gene tested, of which the *sul3* gene was the most frequently-detected genes (42%). Resistance to spectinomycin and streptomycin was mediated by *aadA1* in the majority of the spectinomycin- (68%)

Table 2. Prevalence of resistance genes in relation to resistance phenotypes and distribution of *S. enterica* serovar from poultry (*n*=84) and swine (*n*=104)

Resistance phenotype (<i>n</i>)	Resistance genes	No. of isolates (%) ^{a)}		Serovar ^{b)} (no. of isolates) ^{c)}
		Poultry	swine	
Ampicillin (103)	<i>bla</i> _{PSE1}	4 (5)	2 (2)	Alb(5), Kin(1)
	<i>bla</i> _{TEM}	19 (23)	42 (40)	Alt, Ana(2), Bov, Bsi(10), Cor(2), Eme, Ent (6), Giv, Ked(6), Ken(2), Ori(2), Ris(5), Sen(2), Sta(8), subI(3), Tho, Typ(2), Wel (3), Wor(2)
	both	13 (15)	16 (15)	Alt, Epp, Ked, Sch, Sta (12), subI(4), Typ (8), Wel
Chloramphenicol (59)	at least one	36 (43)	60 (58)	All inclusive strains
	<i>catB</i>	0 (0)	1 (1)	subI
	<i>cmIA</i>	7 (8)	30 (29)	Alt(2), Bsi(8), Giv, subI(3), Ked(8), Sta(14), Wel
Gentamicin (26)	<i>aadB</i>	7 (8)	16 (15)	Ana, Epp, Ked(9), Ken, Sch, subI(5), Sta, Typ(3), Wel
Spectinomycin (103)	<i>aadA1</i>	14 (17)	33 (32)	Alb, Alt(2), Bsi(12), Bov, Cor, Eme, Ent, Ken, Par, Poo, Ris(4), Sch, Sen, Sta(11), subI(4), Typ, Vic(2), Wel
	<i>aadA2</i>	0 (0)	2 (2)	Sta(2)
	both	6 (7)	17 (16)	Ana, Epp, Giv, Ked(8), Ris, Sta(4), subI(3), Typ(3), Wel
Streptomycin (127)	at least one	20 (24)	52 (50)	All inclusive strains
	<i>aadA1</i>	9 (11)	8 (8)	Alb, Eme, Par, Ris(3), Sen, Sta, subI(3), Typ(5), Vic
	<i>strA, strB</i>	9 (11)	12 (12)	Alt, Bsi, Cor(5), Ken, Ori, Poo, Ris(3), Sen, Sta, Tho, Wel(2), Wor(3)
	<i>aadA1, aadA2</i>	2 (2)	8 (8)	Ked(4), Ris, subI(2), Typ(2), Wel
	<i>strA, strB, aadA1</i>	7 (8)	30 (29)	Alt(2), Bov, Bsi(12), Cor, Poo, Ris(2), Sta(14), Typ(2), Vic, Wel
	<i>strA, strB, aadA2</i>	0 (0)	2 (2)	Sta(2)
	<i>strA, strB, aadA1, aadA2</i>	3 (4)	3 (3)	Giv, subI, Sta(4)
	at least one	30 (36)	63 (61)	All inclusive strains
Sulfamethoxazole (139)	<i>sul1</i>	10 (12)	8 (8)	Alb(5), Ana, Eme(2), Kin, Mad, Ori, Par, Ris, Sch, subI(2), Typ, Wel
	<i>sul2</i>	8 (10)	9 (9)	Ago, Cor(6), Ent, Ori, Ris, Sta, Typ(6)
	<i>sul3</i>	7 (8)	30 (29)	Alt(3), Ana(3), Bov, Bsi(11), Ent, Ked(6), Sta(5), Tho, Typ(3), Wel(2), Wor
	all three	2 (2)	7 (7)	Ked, Sta, subI(5), Typ, Wel
	<i>sul1, sul2</i>	0 (0)	2 (2)	Typ(2)
	<i>sul1, sul3</i>	9 (11)	5 (5)	Giv, Epp, Ked(2), Ken, Ris, Sta(6), subI, Wel
	<i>sul2, sul3</i>	0 (0)	8 (8)	Bsi(2), Sta(5), Wel
	at least one	36 (43)	69 (66)	All inclusive strains
Tetracycline (122)	<i>tetA</i>	13 (15)	47 (45)	Ago, Alb, Ana(3), Blo, Bov, Bsi(5), Cor(3), Epp, Giv, Ked(5), Ken, Mad, Ris(7), Sch, Sta(15), subI(5), Typ(5), Wel(2), Wor
	<i>tetB</i>	1 (1)	4 (4)	Ori, Typ(3), Vig
	both	6 (7)	8 (8)	Par, Ked(4), Sta(4), subI, Typ(3), Wel
	at least one	20 (24)	59 (57)	All inclusive strains
	<i>dfrA1</i>	6 (7)	1 (1)	Alb(5), Eme, Mad
	<i>dfrA10</i>	1 (1)	1 (1)	Kin, Wel
Trimethoprim (69)	<i>dfrA12</i>	9 (11)	19 (18)	Ana, Giv, Ken, Par, Ris(3), Sch, Sta(8), subI(4), Typ(5), Wel, Wor(2)
	<i>dfrA10, dfrA12</i>	0 (0)	1 (1)	subI
	at least one	16 (19)	22 (21)	All inclusive strains

a) Some of *bla*_{PSE-1} (2, 2%), *aadA2* (18, 17%), *dfrA1* (8, 12%) and *dfrA12* (16, 23%) were the inserted gene cassettes in class 1 integrons as previously observed [13].

b) Abbreviations: Alb, Albany; Alt, Altona; Ago, Agona; Ams, Amsterdam; Ana, Anatum; Bar, Bareilly; Bov, Bovismorbifican; Bsi, Bsilla; Blo, Blockley; Cor, Corvallis; Ent, Enteritidis; Eme, Emek; Epp, Eppendorf; Giv, Give; Ked, Kedougou; Kin, Kingston; Ken, Kentucky; Mad, Madjorio; Ori, Orion; Pan, Panama; Par, Paratyphi B2; Poo, Poona; Ris, Rissen; Sta, Stanley; subI, subspecies I; Sch, Schwarzengrund; Sen, Senftenberg; Sin, Singapore; Typ, Typhimurium; Tho, Thomson; Vig, Virginia; Vic, Virchow; Wor, Worthington; and Wel, Weltevreden.

c) Serovars represented by only one isolate are shown without number.

and streptomycin- (56%) resistant isolates. The presence of different genes within the same strains, encoding resistance to the same antimicrobials, was detected in 53%.

Sequence analysis of the QRDRs of the *gyrA*, *gyrB*, *parC*,

and *parE* genes revealed that only 2 isolates harbored a point mutation. Replacement of C248 with G leading to a Ser-83-Phe substitution in GyrA was identified in a serovar Senftenberg isolate and a point mutation C-170-G leading to

Table 3. Distribution of resistance genes in relation to antimicrobial resistance patterns ($n=184$)

Antibiotic resistance pattern ^{a)}	No. of isolates (%)	Resistance gene (No.)
SPC	3 (1.6)	—
STR	4 (2.2)	<i>strA</i> (1), <i>strB</i> (1)
SUL	9 (4.9)	<i>sul2</i> (1)
TET	6 (3.3)	<i>tetA</i> (1)
AMP, STR	4 (2.2)	<i>bla</i> _{TEM} (1), <i>strA</i> (2), <i>strB</i> (2)
AMP, SUL	3 (1.6)	<i>bla</i> _{TEM} (2), <i>sul3</i> (1)
SPC, TET	2 (1.1)	<i>tetA</i> (1)
STR, SUL	4 (2.2)	<i>aadA1</i> (1), <i>strA</i> (2), <i>strB</i> (2), <i>sul2</i> (1)
STR, TET	5 (2.7)	<i>aadA1</i> (1), <i>strA</i> (2), <i>strB</i> (2), <i>tetA</i> (1), <i>tetB</i> (1)
AMP, SPC, SUL	5 (2.7)	<i>aadA1</i> (1), <i>bla</i> _{PSE-1} (1), <i>bla</i> _{TEM} (4), <i>sul3</i> (3)
SPC, STR, SUL	8 (4.3)	<i>aadA1</i> (4), <i>dfrA1</i> (1), <i>strA</i> (3), <i>strB</i> (3), <i>sul1</i> (3), <i>sul2</i> (1), <i>sul3</i> (4)
SPC, STR, TET	5 (2.7)	<i>aadA1</i> (4), <i>strA</i> (4), <i>strB</i> (4), <i>tetA</i> (1)
STR, SUL, TET	4 (2.2)	<i>strA</i> (3), <i>strB</i> (3), <i>sul2</i> (4), <i>tetA</i> (3)
SUL, TET, TRI	5 (2.7)	<i>dfrA12</i> (1), <i>sul2</i> (4), <i>tetA</i> (3), <i>tetB</i> (3)
AMP, TET, STR	2 (1.1)	<i>aadA1</i> (1), <i>bla</i> _{PSE-1} (2), <i>bla</i> _{TEM} (2), <i>strA</i> (2), <i>strB</i> (2), <i>tetA</i> (1)
AMP, STR, SUL	3 (1.6)	<i>aadA1</i> (2), <i>bla</i> _{PSE-1} (2), <i>bla</i> _{TEM} (3), <i>strA</i> (1), <i>strB</i> (1), <i>sul3</i> (3)
AMP, STR, SUL, TET	4 (2.2)	<i>aadA1</i> (1), <i>bla</i> _{TEM} (4), <i>strA</i> (2), <i>strB</i> (2), <i>sul2</i> (2), <i>sul3</i> (3)
AMP, SPC, SUL, TET	2 (1.1)	<i>bla</i> _{TEM} (1), <i>sul3</i> (2), <i>tetA</i> (1)
AMP, SPC, STR, SUL	2 (1.1)	<i>aadA1</i> (2), <i>bla</i> _{TEM} (2), <i>strA</i> (1), <i>strB</i> (1), <i>sul2</i> (1), <i>sul3</i> (2)
CHP, SUL, TET, TRI	2 (1.1)	—
CHP, SPC, STR, SUL	2 (1.1)	<i>aadA1</i> (1), <i>cmlA</i> (1), <i>strA</i> (1), <i>strB</i> (1), <i>sul3</i> (1)
SPC, STR, SUL, TRI	2 (1.1)	<i>aadA1</i> (1), <i>dfrA1</i> (1), <i>dfrA10</i> (1), <i>dfrA12</i> (1), <i>strA</i> (1), <i>strB</i> (1), <i>sul1</i> (2), <i>sul2</i> (1), <i>sul3</i> (1)
SPC, STR, SUL, TET	4 (2.2)	<i>aadA1</i> (1), <i>strA</i> (2), <i>strB</i> (2), <i>sul2</i> (2), <i>tetA</i> (1)
AMP, SPC, STR, SUL, TET	4 (2.2)	<i>aadA1</i> (2), <i>bla</i> _{PSE-1} (1), <i>bla</i> _{TEM} (4), <i>strA</i> (3), <i>strB</i> (3), <i>sul1</i> (2), <i>sul2</i> (2), <i>tetA</i> (2), <i>tetB</i> (1)
SPC, STR, SUL, TET, TRI	3 (1.6)	<i>aadA1</i> (2), <i>dfrA10</i> (1), <i>dfrA12</i> (2), <i>sul1</i> (3), <i>tetA</i> (3), <i>tetB</i> (1)
AMP, CHP, SPC, STR, SUL, TET	12 (6.5)	<i>aadA1</i> (10), <i>aadA2</i> (4), <i>bla</i> _{PSE-1} (5), <i>bla</i> _{TEM} (12), <i>cmlA</i> (12), <i>strA</i> (11), <i>strB</i> (11), <i>sul3</i> (12), <i>tetA</i> (9)
AMP, CHP, STR, SUL, TET, TRI	4 (2.2)	<i>aadA1</i> (1), <i>bla</i> _{PSE-1} (3), <i>bla</i> _{TEM} (1), <i>cmlA</i> (1), <i>dfrA1</i> (3), <i>strA</i> (1), <i>strB</i> (1), <i>sul1</i> (3), <i>sul2</i> (1), <i>sul3</i> (1), <i>tetA</i> (1)
AMP, SPC, STR, SUL, TET, TRI	6 (3.3)	<i>aadA1</i> (4), <i>aadA2</i> (1), <i>bla</i> _{PSE-1} (2), <i>bla</i> _{TEM} (6), <i>dfrA12</i> (4), <i>strA</i> (6), <i>strB</i> (6), <i>sul1</i> (5), <i>sul2</i> (2), <i>sul3</i> (4), <i>tetA</i> (1)
AMP, CHP, SPC, STR, TET, TRI	2 (1.1)	<i>aadA1</i> (2), <i>bla</i> _{PSE-1} (1), <i>bla</i> _{TEM} (2), <i>cmlA</i> (1), <i>dfrA12</i> (1), <i>strA</i> (2), <i>strB</i> (2), <i>sul1</i> (1), <i>sul3</i> (1), <i>tetA</i> (2)
AMP, GEN, STR, SUL, TET, TRI	3 (1.6)	<i>aadA1</i> (2), <i>aadB</i> (3), <i>bla</i> _{PSE-1} (2), <i>bla</i> _{TEM} (3), <i>strA</i> (1), <i>strB</i> (1), <i>sul2</i> (1), <i>tetB</i> (1)
AMP, GEN, SPC, SUL, TET, TRI	3 (1.6)	<i>aadA1</i> (3), <i>aadA2</i> (1), <i>aadB</i> (3), <i>bla</i> _{PSE-1} (2), <i>bla</i> _{TEM} (3), <i>dfrA12</i> (2), <i>sul1</i> (3), <i>sul3</i> (2), <i>tetA</i> (3)
AMP, CHP, GEN, SPC, STR, SUL, TET	6 (3.3)	<i>aadA1</i> (6), <i>aadA2</i> (6), <i>aadB</i> (6), <i>bla</i> _{TEM} (6), <i>cmlA</i> (6), <i>strA</i> (2), <i>strB</i> (2), <i>sul1</i> (3), <i>sul2</i> (1), <i>sul3</i> (6), <i>tetA</i> (6), <i>tetB</i> (2)
AMP, CHP, SPC, STR, SUL, TET, TRI	11 (6.0)	<i>aadA1</i> (10), <i>aadA2</i> (1), <i>bla</i> _{PSE-1} (5), <i>bla</i> _{TEM} (9), <i>cmlA</i> (8), <i>dfrA10</i> (1), <i>dfrA12</i> (5), <i>strA</i> (9), <i>strB</i> (9), <i>sul1</i> (6), <i>sul2</i> (3), <i>sul3</i> (9), <i>tetA</i> (8), <i>tetB</i> (3)
AMP, GEN, SPC, STR, SUL, TET, TRI	2 (1.1)	<i>aadA1</i> (2), <i>aadA2</i> (2), <i>aadB</i> (1), <i>bla</i> _{PSE-1} (1), <i>bla</i> _{TEM} (1), <i>dfrA12</i> (2), <i>strA</i> (1), <i>strB</i> (1), <i>sul1</i> (2), <i>sul2</i> (1), <i>tetA</i> (2)
AMP, CHP, GEN, SPC, STR, SUL, TET, TRI	6 (3.3)	<i>aadA1</i> (6), <i>aadA2</i> (6), <i>aadB</i> (4), <i>bla</i> _{PSE-1} (4), <i>bla</i> _{TEM} (6), <i>cmlA</i> (4), <i>dfrA12</i> (6), <i>strA</i> (2), <i>strB</i> (2), <i>sul1</i> (6), <i>sul2</i> (5), <i>sul3</i> (5), <i>tetA</i> (6), <i>tetB</i> (1)

a) Abbreviations: AMP, ampicillin; CHP, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; SPC, spectinomycin; STR, streptomycin; SUL, sulfamethoxazole; TET, tetracycline; TRI, trimethoprim.

a Thr-57-Ser substitution in ParC was found in the other serovar Senftenberg isolate. No mutations were observed in the QRDRs of the ciprofloxacin sensitive control.

Antimicrobial resistance patterns and resistance gene profiles are shown in Table 3. All the strains harboring more than one resistance gene were resistant to three or more antimicrobials. Statistical analysis showed that there were significant associations between being resistance to an antimicrobial agent and having resistance gene for all genes

($p<0.01$) except *catB* and *dfrA10*, which were not significantly associated with being resistance to chloramphenicol and trimethoprim respectively ($p=0.051$). Having more than one gene conferring resistance to a single agent was significantly associated with increasing MIC level and proportion of resistance isolates ($p<0.01$). In addition, having genes conferring resistance to multiple drugs was significantly associated with being multiresistance ($p<0.01$). Twenty-two percent of the *Salmonella* isolates were nega-

tive to all the resistance genes tested, even through most of these isolates were resistant to at least one antimicrobial agent (data not shown).

DISCUSSION

Our findings showed that most of the antimicrobial resistance genes tested were found at high rates, indicating that these genes play an important role in conferring resistance among the *Salmonella* isolates in this study. This data also confirmed the wide diversity of mechanisms mediating antimicrobial resistance in different *Salmonella* serovars of animal origins. These investigated resistance genes were previously observed at high rate in *Salmonella* from humans, food animals and food products [1, 4, 19].

As class 1 integrons were previously found to be prevalent in the resistant *Salmonella* isolates of our collection [15], integrons of either class 2 and 3 were not identified in this study. Several reports showed that class 1 integrons were more frequently observed than class 2 integrons [19, 28] and the rarely described class 3 integrons are to date not identified in this pathogen [28]. These results once again highlight the important role of class 1 integrons in dissemination of antimicrobial resistance.

Resistance to quinolones in *Salmonella* is usually a result of a single point mutation in the QRDR of the *gyrA* gene (nucleotides 67 to 122) [12], which is consistent with the results in this study. The mutation Ser-83-Phe and Thr-57-Ser had been previously described [9, 10, 17]. However, a ciprofloxacin-resistant strain did not have mutations in *gyrA*, *gyrB*, *parC*, and *parE*. The possible resistance mechanisms for the isolates could be decreased permeability of the outer membrane, an active efflux [5, 12] or the existence of as yet unknown additional resistance mechanisms. These results warrant further investigation.

Up to 53% of the isolates harbored 2 or 3 different resistant genes for the same antimicrobials, such as *sul1*, *sul2* and *sul3* for sulphonamide resistance or *dfrA10* and *dfrA12* for trimethoprim resistance. These included all of the strains that contained class 1 integrons. For these class 1 integron-positive strains, it is possible that one resistance gene was associated with the integrons and gene clusters on SGI1 variants, but the other gene(s) were located in a plasmid [26]. However, some of the class 1 integrons-negative strains also carried more than one resistance gene for the same antimicrobial resistance. Further studies to characterize plasmids and elucidate the alternative possibilities are required.

The prevalence of chloramphenicol resistance and the existence of the chloramphenicol resistance gene (s) among *E. coli* isolates has been demonstrated [2, 27] even through the antibiotic has been prohibited for using in domestic animals. This is in agreement of the results in our study, which the *cmlA* gene was found in the majority of chloramphenicol-resistant isolates. In our previous study, the *cmlA* gene in 7 chloramphenicol-resistant *Salmonella* strains was shown to be part of gene cassettes in class 1 integrons with

unusual 3' conserved segment linked to the *qacH-sul3* domain and the common resistance genes in the gene cassettes included *aadA1* and *aadA2*, and *qacH* [7]. This close-genetic linkage to other resistance genes could be a mechanism for maintenance and co-selection of chloramphenicol resistance in *Salmonella* in the absence of chloramphenicol selection pressure [2, 27].

The present study demonstrated good correlations between the presence of antimicrobial resistance genes and corresponding resistance phenotype suggesting that these resistance genes were usually expressed when present. For each antimicrobial agent, the MIC level of the *Salmonella* isolates carrying more than one corresponding-resistance gene was higher than that of the strains with only a single gene (data not shown), indicating the synergy among the genes in mediating resistance. However, these results did not rule out that other resistance genes not tested in this study are also present. Several resistant-*Salmonella* isolates did not carry the investigated resistance genes, indicating that there must be other mechanisms, which contributed to such resistance and were not tested in this study e.g. active efflux and other inactivating enzymes.

In conclusion, the results in this study provide useful information regarding the dissemination of antimicrobial resistance genes among a wide range of the resistant *Salmonella* serovars confirming an important role of food animals in the spread of antimicrobial resistance. Antimicrobial-resistant bacteria and their resistance determinants are considered hazards in antimicrobial resistance risk assessment; therefore, the information obtained could be used as part of risk analysis of the distribution and development of antimicrobial resistance. However, data regarding the epidemiological situations of antimicrobial resistance and mechanism basis of resistance is still limited especially in most developing countries. Continued monitoring of antimicrobial resistance in a huge strain collection of *Salmonella* along the food chain is required so that comparison of antimicrobial resistance from the different origins could be effectively performed. Guidelines on the prudent use of antimicrobials in animal production should be developed and readily accessible. The guidelines should base on existing regulation, resistance situation and human health concerns and include recommendations on a rational antimicrobial treatment of choice.

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