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Original Article

Prevalence and antimicrobial resistance of *Salmonella* isolates from goose farms in Northeast China

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Abstract

Background: *Salmonella* is one of the most important enteric pathogenic bacteria that threatened poultry health. **Aims:** This study aimed to investigate the prevalence and antimicrobial resistance of *Salmonella* isolates in goose farms. **Methods:** A total of 244 cloacal swabs were collected from goose farms to detect *Salmonella* in Northeast China. Antimicrobial susceptibility, and resistance gene distribution of *Salmonella* isolates were investigated. **Results:** Twenty-one *Salmonella* isolates were identified. Overall prevalence of *Salmonella* in the present study was 8.6%. Among the *Salmonella* isolates, the highest resistance frequencies belonged to amoxicillin (AMX) (85.7%), tetracycline (TET) and trimethoprim/sulfamethoxazole (SXT) (81%), followed by chloramphenicol (CHL) (76.2%), florfenicol (FLO) (71.4%), kanamycin (KAN) (47.6%), and gentamycin (GEN) (38.1%). Meanwhile, only 4.8% of the isolates were resistant to ciprofloxacin (CIP) and cefotaxime (CTX). None of the isolates was resistant to cefoperazone (CFP) and colistin B (CLB). Twenty isolates (95%) were simultaneously resistant to at least two antimicrobials. Ten resistance genes were detected among which the *bla*$_{\text{TEM-1}}$, *cmlA*, *aac(6')-Ib-cr*, *sul1*, *sul2*, *sul3*, and *mcr-1.1* were the most prevalent, and presented in all 21 isolates followed by *tetB* (20/21), *qnrB* (19/21), and *floR* (15/21). **Conclusion:** Results indicated that *Salmonella* isolates from goose farms in Northeast China exhibited multi-drug resistance (MDR), harboring multiple antimicrobial resistance genes. Our results will be useful to design prevention and therapeutic strategies against *Salmonella* infection in goose farms.

Key words: Antimicrobial resistance, Goose, Resistance gene, *Salmonella*

Introduction

Salmonella genus belongs to the Enterobacteriaceae family which is a Gram-negative, rod-shaped bacterium. *Salmonella* is responsible for a series of diseases such as salmonellosis, a variety of illnesses including gastroenteritis, which threats animal and human public healths (Amajoud *et al*., 2017). In poultry, *Salmonella* is one of the most important genera of enteric pathogenic bacteria (Guard *et al*., 2010). Many of *Salmonella* serotypes can reserve in poultry and cause significant morbidity, mortality, and economic losses. More importantly, poultry can carry a significant number of *Salmonella* without showing any clinical signs of infection, and bring a potential threat to the bird's health. Therefore, the prevention of *Salmonella* infection is necessary for poultry rearing process.

Resistance to antimicrobial agents can occur in many bacterial species where the pathogen might alter its permeability to the antimicrobial substances, degrade the antimicrobial effects, cause its efflux or lead to its inactivation by enzymatic means (Livermore, 2003). In the past decades, the use of antimicrobial agents has been considered the most important way to treat and control *Salmonella* infection. However, the widespread utilization of antimicrobial agents increases antimicrobial resistance and emerges multi-drug resistance (MDR) of *Salmonella* serotypes (Lamas *et al*., 2016). Arising MDR in *Salmonella*, not only occurs against the front-line antimicrobials, such as chloramphenicol (CHL) and trimethoprim/sulfamethoxazole (SXT), but also happens against clinically important antimicrobial agents, such as β-lactams and fluoroquinolones, leading to treatment failure of animals and human (Lunguya *et al*., 2013). Resistance to antimicrobial substances can occur via resistance genes. Constant selective pressure by antimicrobial overuse increases the prevalence rates of resistance genes in bacteria. Identifying these gene patterns will be helpful to control excessive antibiotic usage.

Goose is well known for its strong adaptability, rapid growth, rich nutrient content and low input requirement. China has the largest goose production in the world. In Northeast China, the goose industry is important to improve the economic benefits of local farmers. However, geese are more frequently reared in semi-intensive housing systems using simple accommodations with access to outside pens and bathing water. Therefore, *Salmonella* can be introduced to goose flocks from multiple sources such as environment, feed, and vectors

due to a lack of efficient biosecurity measures. *Salmonella* infection in goose farms probably causes huge economic loss. To the best of our knowledge, few studies have been devoted in prevalence and antimicrobial resistance of goose-originated *Salmonella* in Northeast China. Herein, ten goose farms located in Liaoning and Jilin province, Northeast China, were selected to sample, isolate and identify *Salmonella* through selective medium culturing, biochemical testing, and molecular biologic identification. Subsequently, the antimicrobial resistance pattern and resistance gene distribution of *Salmonella* isolates were investigated using disk diffusion and polymerase chain reaction (PCR) methods. The finding of this study will be helpful to prevent and control *Salmonella* infection in goose farms efficently.

Materials and Methods

Ethics statement

Experimental procedures were approved by the Animal Welfare Committee at the College of Animal Science and Veterinary Medicine of Shenyang Agricultural University (No. 2011036).

Sample collection

From April 2016 to July 2017, 244 cloacal swabs were collected randomly from ten individual goose farms located in Liaoning and Jilin province. All samples were transported to a laboratory in an insulated ice chest containing ice packs within 3 h for further bacteriological analysis.

Isolation and identification of *Salmonella*

Salmonella was isolated using the Chinese National Standard Method (GB 4789.4-2010) with some modifications (Yang *et al*., 2019). Briefly, cloacal swabs were placed into sterile 10 ml plastic tubes containing 5 ml of buffered peptone water (BPW, QingDao Hopebio-Technology Co., Ltd., Qindao, China) and incubated at 37°C for 8 h. Approximately 1 ml of pre-enrichment culture was then incubated into 10 ml of selenite cysteine broth (SC, QingDao Hopebio-Technology Co., Ltd., Qindao, China) at 37°C for 24 h. A loop of inoculum from the SC culture was streaked onto selective culture medium *Salmonella shigella* agar (SS-Agar, QingDao Hopebio-Technology Co., Ltd., Qindao, China) plates, and incubated at 37°C for 24 h. Colorless colonies with black centers on the SS culture plates were presumed as *Salmonella* colonies selected for further identification including biochemical, and molecular assays.

Biochemical tests comprised triple sugar iron slant reaction, indole reaction, urease test, lysine decarboxylase, potassium cyanide, mannose, sorbitol, and o-Nitrophenyl-β-D-galactopyranoside (ONPG) tests (Beijing Land Bridge Technology Co., Ltd., Beijing, China).

Molecular assay was allocated to confirm suspected colonies, followed by biochemical tests, by PCR amplification of *invA* gene using primers *invA*-F (5´-GTC CTC CGC CCT GTC TAC-3´) and *invA*-R (5´-TCC TAA CGA CGA CCC TTC-3´) (Rahn *et al*., 1992). The expected-size PCR products were sequenced by Sangon Biotech Co., Ltd., and aligned using the basic logical alignment search tool (BLAST) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The identified isolates were stored in Luria-Bertani (LB) broth containing 15% glycerol at -80°C.

Antimicrobial susceptibility testing

All identified *Salmonella* isolates were tested for antimicrobial susceptibility using the standard Kirby-Bauer disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012). The following antimicrobial disks including cefoperazone (CFP), cefotaxime (CTX), amoxicillin (AMX), CHL, florfenicol (FLO), gentamycin (GEN), kanamycin (KAN), norfloxacin (NOR), ciprofloxacin (CIP), tetracycline (TET), SXT, and colistin B (CLB) were purchased from Hangzhou Microbial Reagent Co., Ltd. *Escherichia coli* (ATCC No. 25922) was used as a quality control strain. The isolates resistant to two or more different antimicrobials categories were defined as MDR (Magiorakos *et al*., 2012).

Detection of antimicrobial resistance genes

Based on antimicrobial resistance profiles, all *Salmonella* isolates were examined for the presence of resistance genes using the PCR method. Twenty-seven primer pairs were designed to target twenty-seven antimicrobial resistance genes that confer resistance to seven categories of antimicrobial agents (Table 1). Bacterial DNA was extracted using Rapid Bacterial Genomic DNA Isolation kit (Sangon Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Polymerase chain reaction was conducted in a final volume of 25 µL containing 2.5 µL of template DNA, 1 µL of each primer (10 µM), 12.5 µL Taq PCR Mastermix (TIANGEN Biotech Co., Ltd., Beijing, China), and 8 µL of double-distilled water. The PCR cycling conditions consisted of an initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 95°C for 45 s, annealing at their respective annealing temperatures for 45 s, and extension at 72°C for 1 min. The expected-size PCR products were sequenced by Sangon Biotech Co., Ltd. (Shanghai, China). The DNA sequence data were compared with the GenBank database using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Prevalence of *Salmonella* isolates

A total of 21 *Salmonella* isolates were obtained from 244 cloacal swabs collected from ten goose farms located in Liaoning and Jilin province of Northeast China. The overall prevalence of *Salmonella* in this study was 8.6%. Table 2 shows the prevalence details of *Salmonella* per geese farm; one isolate was detected in Farm B in Taian city, seven isolates were isolated from

Table 1: Primers used for detection of genes encoding resistance to different antimicrobials

Antimicrobial (s)	Resistance gene	Primer sequences	Size (bp)	Annealing temperature (°C)	Referenced GenBank accession No.
β-lactams	bla_{TEM-1}	F: CAGCGGTAAGATCCTTGAGA R: ACTCCCCGTCGTGTAGATAA	643	54	MG063853.1
	bla_{SHV}	F: TGACGGTCGGCGAACTCT R: ATTGGCGGCGCTGTTATC	759	58	HQ625487.1
	bla_{CTX-M}	F: TTTGCGATGTGCAGTACCAGTAA R: CGATATCGTTGGTGGTGCCATA	544	57	NG_049000.1
	bla_{CMY-2}	F: TGGCCGTTGCCGTTATCTAC R: CCCGTTTTATGCACCCATGA	870	56	LC216401.1
Aminoglycosides	*aac(3)-IIa*	F: CGGCCTGCTGAATCAGTTTC R: AAAGCCCACGACACCTTCTC	439	57	NG_047250.1
	aac(3)-IV	F: GTCTCTGACACATTCTGGCG R: ATGACCGACTGGACCTTC	387	55	X01385
	aacC2	F: GGCAATAACGGAGGCAATTCGA R: CTCGATGGCGACCGAGCTTCA	450	60	X51534
	Kn	F: ACTGGCTGCTATTGGGCGA R: CGTCAAGAAGGCGATAGAAGG	515	58	U66885
	aph(3')-IIa	F: TCCGGTGCCCTGAATGAACT R: ACGGGTAGCCAACGCTATGT	519	59	NG_047417.1
	aadB	F: GGCAGACGAAGCGTATGAA R: CGACCTGAAAGCGGCAC	244	56	HQ832474.1
Tetracycline	*tetA*	F: GCGCCTTTCCTTTGGGTTCT R: CCACCCGTTCCACGTTGTTA	831	55	AY150213.1
	tetB	F: CCCAGTGCTGTTGTTGTCAT R: CCACCACCAGCCAATAAAAT	723	54	JN676152.1
	tetC	F: GTCACTATGGCGTGCTGCTA R: TCTCCCTTATGCGACTCCTG	436	57	DQ205473.1
Sulfonamide	*sul1*	F: TCACCGAGGACTCCTTCTTC R: CAGTCCGCCTCAGCAATATC	331	56	EF427691.1
	sul2	F: CGGCATCGTCAACATAACC R: CTGACTTCATCCGCACAC	722	62	NG_048111.1
	sul3	F: AGATGTGATTGATTTGGGAGC R: TAGTTGTTTCTGGATTAGAGCCT	443	52.5	AY316203.1
Chloramphenicol	*catA1*	F: GCAAGATGTGGCGTGTTACGGTGAA R: TCATTAAGCATTCTGCCGACATGGA	258	60	EF523685.1
	catA2	F: GAACACTTTGCCCTTTATCGTC R: TCCTGCTGAAACTTTGCCATCGT	482	56	NG_047596.1
	catA3	F: TGATGAGTTGAGAATGGCGATA R: GAGAGCGGCAATAACAGTCTA	358	54	X07848
	cmlA	F: GCGGGCTATCTTTGCGTTTC R: AAGTAGACTGCCGTGACCGTTCC	540	59	DQ205477.1
	floR	F: TCCTGAACACGACGCCCGCTAT R: TCACCGCCAATGTCCCGACGAT	960	64	MH607134.1
Fluoroquinolones	*qnrA*	F: TTCAGCAAGAGGATTTCTCA R: GGCAGCACTATTACTCCCAA	500	52	AY070235
	qnrB	F: CCTGAGCGGCACTGAATTTAT R: GTTTGCTGCTCGCCAGTCGA	671	68	MF615305.1
	qnrS	F: CAATCATACATATCGGCACC R: TCAGGATAAACAACAATACCC	420	50	FJ418153.1
	aac(6')-Ib-cr	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	482	58.5	EU543272
	qepA	F: CCAGCTCGGCAACTTGATAC R: ATGCTCGCCTTCCAGAAAA	500	55	NG_062251.1
Polypeptides	*mcr-1.1*	F: CTTGGTCGGTCTGTAGGG R: CGGTCAGTCCGTTTGTTC	334	54	LT159976.1

Farm E in Shenyang city, two isolates were isolated from Farm F in Anshan city, one isolate was characterized in Farm I in Jilin city, and ten isolates were isolated from the Farm J in Siping city. The prevalence of positive samples was 3.3, 23.3, 6.7, 5.0, and 50% in Farm B, E, F, I, and J, respectively; while no *Salmonella* was isolated from Farm A, C, D, G, and H.

Antimicrobial resistance of *Salmonella* isolates

All 21 *Salmonella* isolates were tested for susceptibility against twelve antimicrobial agents, possessing great importance in veterinary practice. The results are summarized in Table 3. High resistance rates were observed against AMX (85.7%), TET (81%), and SXT (81%), followed by CHL (76.2%), FLO (71.4%), KAN (47.6%), and GEN (38.1%). Lower levels of

Table 2: Prevalence of *Salmonella* isolated from goose farms in Liaoning and Jilin provinces of Northeast China

Sample source	Number of samples	Positive samples	Percentage of positive samples
A (Panjin)	20		0
B (Taian)	30	B30	3.3% (1/30)
C (Tieling)	24		0
D (Liaoyang)	30		0
E (Shenyang)	30	E13, E14, E15, E22, E24, E27, E29	23.3% (7/30)
F (Anshan)	30	F23, F27	6.7% (2/30)
G (Dehui)	20		0
H (Yushu)	20		0
I (Jilin)	20	I17	5% (1/20)
J (Siping)	20	J2, J4, J6, J8, J10, J12, 14, J16, J17, J19	50% (10/20)

Table 3: Antimicrobial resistance profiles of *Salmonella* isolates from goose farms in Liaoning and Jilin provinces of Northeast China

Antimicrobial	Resistant isolates		Intermediate isolates		Sensitive isolates	
	Number	%	Number	%	Number	%
β-Lactams						
AMX	18	85.7	1	4.8	2	9.5
CFP	0	0	2	9.5	19	90.5
CTX	1	4.8	1	4.8	19	90.5
Chloramphenicol						
CHL	16	76.2	2	9.5	3	14.3
FLO	15	71.4	3	14.3	3	14.3
Aminoglycosides						
GEN	8	38.1	2	9.5	11	52.4
KAN	10	47.6	2	9.5	9	42.9
Quinolones and fluoroquinolone						
NOR	4	19	6	28.6	11	52.4
CIP	1	4.8	9	42.9	11	52.4
Tetracycline						
TET	17	81	2	9.5	2	9.5
Sulfonamides						
SXT	17	81	0	0	4	19
Polypeptide						
CLB	0	0	0	0	21	100

AMX: Amoxicillin, CFP: Cefoperazone, CTX: Cefotaxime, CHL: Chloramphenicol, FLO: Florfenicol, GEN: Gentamycin, KAN: Kanamycin, NOR: Norfloxacin, CIP: Ciprofloxacin, TET: Tetracycline, SXT: Trimethoprim/sulfamethoxazole, and CLB: Colistin B

resistance were found for NOR (19%), and CIP (4.8%). All isolates were susceptible to CLB (100%). In addition, 90.5% of the isolates were susceptible or intermediately susceptible to CFP, and CTX. One isolate did not show resistance to any of the twelve tested antimicrobial agents.

As shown in Table 4, among all 21 *Salmonella* isolates, 20 isolates exhibited MDR (95%). Especially, one isolate displayed resistance to eight antimicrobials, and eight isolates showed resistance to seven antimicrobials. Totally, thirteen MDR patterns were observed. The most frequent MDR pattern was SXT-FLO-CHL-TET-KAN-GEN-AMX (n=6), followed by SXT-NOR-FLO-CHL-TET-KAN-AMX (n=2), and FLO-CHL-TET-AMX (n=2).

Distribution of antimicrobial resistance genes

As demonstrated in Table 5, among the 21 *Salmonella* isolates, ten resistance genes belonged to seven categories of antimicrobials including β-lactams, aminoglycosides, TET, CHL, sulfonamide, fluoro-quinolones, and polypeptides. It is noteworthy that the $bla_{\text{TEM-1}}$, *cmlA*, *aac(6')-Ib-cr*, *sul1*, *sul2*, *sul3*, and *mcr-1.1* were the most prevalent resistance genes present in all 21 *Salmonella* isolates, followed by *tetB* (20/21), *qnrB* (19/21), and *floR* (15/21). However, bla_{SHV}, $bla_{\text{CTX-}}$

Table 4: MDR patterns of *Salmonella* isolates from goose farms in Liaoning and Jilin province of Northeast China

MDR patterns	Total number of Salmonella isolates
SXT-NOR-FLO-CHL-TET-KAN-GEN-AMX	1
SXT-NOR-FLO-CHL-TET-KAN-AMX	2
SXT-FLO-CHL-TET-KAN-GEN-AMX	6
SXT-FLO-CHL-TET-KAN-AMX	1
SXT-NOR-FLO-CHL-TET-AMX	1
SXT-CHL-TET-GEN-AMX	1
SXT-FLO-CHL-TET-AMX	1
SXT-FLO-CHL-AMX	1
FLO-CHL-TET-AMX	2
TET-CTX-AMX	1
SXT-AMX	1
SXT-TET	1
SXT-CIP	1

MDR: Multi-drug resistance, SXT: Trimethoprim/ sulfamethoxazole, NOR: Norfloxacin, FLO: Florfenicol, CHL: Chloramphenicol, TET: Tetracycline, KAN: Kanamycin, GEN: Gentamycin, AMX: Amoxicillin, CTX: Cefotaxime, and CIP: Ciprofloxacin

Table 5: Antimicrobial resistance phenotype and resistance genes of 21 *Salmonella* isolates

Salmonella isolates	Resistance phenotype	Detected resistance genes									
B30	SXT-NOR-FLO-CHL-TET-KAN-GEN-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
E13	FLO-CHL-TET-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*		*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
E14	SXT-FLO-CHL-TET-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
E15	SXT-NOR-FLO-CHL-TET-KAN-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*		*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
E22	SXT-CIP	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*		*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
E24	SXT-NOR-FLO-CHL-TET-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
E27	TET-CTX-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
E29	SXT-CHL-TET-GEN-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
F23	FLO-CHL-TET-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
F27	SXT-FLO-CHL-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
I17	SXT-TET	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*		*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
J2	SXT, NOR, FLO, CHL, TET, KAN, AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
J4	None resistance	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*		*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
J6	SXT-FLO-CHL-TET-KAN-GEN-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
J8	SXT-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*		*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
J10	SXT-FLO-CHL-TET-KAN-GEN-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
J12	SXT-NOR-FLO-CHL-TET-KAN-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*		*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
J14	SXT-FLO-CHL-TET-KAN-GEN-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*		*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
J16	SXT-FLO-CHL-TET-KAN-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
J17	SXT-FLO-CHL-TET-KAN-GEN-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*	*tetB*	bla_{TEM-1}	*mcr-1.1*
J19	SXT-FLO-CHL-TET-KAN-GEN-AMX	*sul1*	*sul2*	*sul3*	*aac(6')-Ib-cr*	*qnrB*	*floR*	*cmlA*		bla_{TEM-1}	*mcr-1.1*

SXT: Trimethoprim/sulfamethoxazole, NOR: Norfloxacin, FLO: Florfenicol, CHL: Chloramphenicol, TET: Tetracycline, KAN: Kanamycin, GEN: Gentamycin, AMX: Amoxicillin, CIP: Ciprofloxacin, and CTX: Cefotaxime

M, bla_{CMY-2}, *aac(3)-IIa*, *aac(3)-IV*, *aacC2*, *Kn* (aminoglycoside 3´-phosphotransferase), *aph(3´)-IIa*, *aadB*, *tet*A, *tet*C, *catA1*, *catA2*, *catA3*, *qnrA*, *qnrS*, and *qepA* genes were not identified in any of the isolates.

Discussion

Salmonella is widely present in intestines of domesticated animals especially poultry, and could easily resist to antimicrobials under antibiotics pressure. It is important to determine the *Salmonella* prevalence and its antimicrobial resistance for devising prevention and treatment strategies in goose farms.

According to our study, the total positive isolation rate of *Salmonella* from goose farms was 8.6% which was less than previous report (12.4%) from some provinces of China (Gong *et al.*, 2014). Our results also revealed various isolation rates from different goose farms. The *Salmonella* emergence may depend on environmental infection or contact with infected human and other animals in rearing area. The difference in isolation rate may be related to the size of goose farms, variation of the environmental conditions, management and sanitation of the different farms. Although our result may not represent the whole goose population in these regions due to the small sample size, it imply the question of sanitary control and the contamination of *Salmonella* in goose farms. Moreover, it represents a serious concern to public health because of this pathogen potential hazard to disseminate from farms to communities.

Our study indicated that 95% of the *Salmonella* isolates were resistant to at least two antimicrobial agents, and 52% of isolates exhibited MDR phenotype to more than 6 antimicrobials simultaneously. There was a high prevalence of resistance to AMX (85.7%), SXT (81%), TET (81%), CHL (76.2%), and FLO (71.4%) that have been extensively used in poultry practices in China, noted by the Chinese Veterinary Pharmacopeia (Wang *et al.*, 2020). Other reports have been also complied with these results in poultry (Shah *et al.*, 2017; Zhao *et al.*, 2017; Phongaran *et al.*, 2019). In addition, it is worth mentioning here that no *Salmonella* isolate was observed to be resistant to CFP and CLB. Cefoperazone is a third-generation cephalosporin with broad-spectrum antimicrobial activity against Gram-positive and Gram-negative organisms. Colistin is prescribed for treatment and prevention of enteric diseases mainly in poultry and pigs. However, it is banned for agriculture purposes in China due to its toxicity (Walsh and Wu, 2016). Currently, the primary antimicrobial treatment options for *Salmonella* infection include fluoroquinolones, and extended-spectrum cephalosporins (Folster *et al.*, 2015). Our study showed that just a few *Salmonella* isolates (4.8%) were resistant to CTX and CIP, and no isolate exhibited resistance to CFP. This result demonstrated that CTX and CIP could be considered for treatment of *Salmonella* infection.

The widespread usage of antibiotics in veterinary practice has caused selective pressure, resulting in an increase in genetic sequences that confer resistance to bacteria. A number of different resistance genes were detected in our study. The bla_{TEM-1} was the most commonly identified β-lactamase gene in *Salmonella* isolates and conferred resistance to AMX (Igbinosa, 2015). The expression of the bla_{CTX-M}, bla_{CMY-2} and bla_{SHV} gene can hydrolyze third and fourth generation cephalosporins (Gonzalez-Sanz *et al.*, 2009). In our study, all AMX resistant isolates carried bla_{TEM-1} gene. None isolate was identified to harbor bla_{CTX-M}, bla_{CMY-2} and bla_{SHV} genes. It showed significant consistency with its resistant phenotype to β-Lactams antibiotics. Resistance to sulfamethoxazole was mediated by the *sul1*, *sul2*, *sul3* gene. Likewise, these genes exist in all *Salmonella* isolates that have exhibited resistance to SXT. Despite CHL is prohibited in domestic animals, the

cmlA gene mediating the CHL resistance was found in all *Salmonella* isolates in our study demonstrating relatively better correlation with its resistance phenotype (76.2%). Florfenicol, a new chemosynthesis broad spectrum antibiotic of CHL analogs, has been widely used in veterinary medicine. The *floR* gene, conferring resistance to this antibiotic, was identified in 71.4% of our *Salmonella* isolates, in consistent with its resistant phenotype. Fluoroquinolones and quinolones are the frequently used antimicrobial agents for treating *Salmonellosis*. The resistance to fluoroquinolones and quinolones was associated with the presence of PMQR (plasmid-mediated quinolone resistance) encoding genes *qnrA*, *qnrB*, *qnrS*, and *aac(6´)-Ib-cr* (Penha Filho *et al*., 2019). In the present study, the co-existence of *qnrB* and *aac(6')-Ib-cr* genes in 90% (19/21) *Salmonella* isolates make a great concern in controling *Salmonella* infections using fluoroquinolones agents.

A discrepancy has been reported between phenotypic and genotypic resistance of isolated bacteria. Some times a resistance gene is present but no phenotypic resistance is observed in bacteria, or vise-versa (Almeida *et al*., 2018). This discrepancy also was observed in our present study. For instance, no aminoglycosides-resistance gene was detected among the *Salmonella* isolates, while 38.1% and 47.6% of isolates showed resistance to GEN and KAN, respectively. The *mcr-1* gene plays an important role in colistin resistance of isolated *Salmonella* (Liu *et al*., 2016). It has been reported to be detected in *Salmonella* isolates from humans, animals, environment, and food in many countries (Yi *et al*., 2017; Lu *et al*., 2019). Even though the prevalence rate of *mcr-1* was found low in hospital *Salmonella* isolates (Cui *et al*., 2017), it remained high in agriculture globally, especially among poultry in China (Nang *et al*., 2019). The *mcr-1* gene has been detected in colistin-susceptible *E. coli* strains (Quan *et al*., 2017) which is also found in all 21 *Salmonella* isolates in our result, nevertheless they were all susceptible to CLB. The reasons caused this discrepancy need to be investigated in future research.

In conclusion, we identified twenty-one *Salmonella* isolates from goose farms in Northeast China. The high frequency of antimicrobial resistance and multiple MDR patterns were observed among these *Salmonella* isolates. All of *Salmonella* isolates harbored multiple antimicrobial resistance genes. This study demonstrated that goose could also be a potential reservoir for *Salmonella*. It is necessary to reinforce the surveillance and to find substitutions for resistant antimicrobial agents to control *Salmonella* infection in goose farms.

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Conflict of interest

All authors do not have conflicts of interest.

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