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Resistance Analysis of *Salmonella enterica* Serovar Indiana to the Chloramphenicol

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Abstract: This study studies chloramphenicol's resistance genes of *Salmonella enterica* serovar Indiana isolated from chicken in China. Salmonella samples were collected from chicken hatcheries, farms and slaughter houses located in Shandong Province. Salmonella was isolated, identified and tested for drug susceptibility. The primers were designed to detect the resistance genes of chloramphenicol by PCR. The results showed that researchers isolated 23.28% strains of *Salmonella enterica* serovar Indiana. Resistance rates to chloramphenicol of *Salmonella enterica* serovar Indiana from hatcheries, farms, slaughter houses were chloramphenicol (50, 83.33, 93.30%), florfenicol (83.33, 100, 100%), thiamphenicol (63, 65, 77%), respectively. During the 80 resistant strains of chloramphenicol, 54 strains were detected *catA1* genes, 74 strains were detected *floR* genes and the 5 strains were detected *cmlA* genes. These result indicated that resistance rate of *Salmonella enterica* serovar Indiana from three sources to chloramphenicol were different. Interestingly, researchers found that 4 isolates carried *catA1*, *floR* and *cmlA* genes from the farms (2), slaughter houses (2) and 49 isolates carried *catA1* and *floR* genes from the hatchery (4), farms (19) and slaughter houses (26).

Key words: *Salmonella enterica* serovar Indiana, chloramphenicol, resistance genes, catA1, floR, cmlA

INTRODUCTION

Salmonella is a non-spore-forming, non-capsulated, Gram-negative bacillus (Zhang *et al*., 2012) with the majority of Salmonella serotypes pathogenic to humans and animals. Salmonella infections occur frequently in poultry and are difficult to control, particularly in Intensive Farming Systems (Penha *et al*., 2009). As a broad-spectrum antibiotic, chloramphenicol has strong antimicrobial effects on Gram-negative bacteria such as Salmonella and *Escherichia coli*. Therefore, wide and extensive use of chloramphenicol drugs in clinical practice has led to Salmonella resistance. Liao *et al*. (2011) reported a rate of 76.9% for pathogenic Salmonella resistance to chloramphenicol while Elmadiena *et al*. (2013) reported a rate of 98.4% in the United States. Monitoring data has shown that pathogenic Salmonella resistance has become a focus of global attention (Zhu, 2001; Zhou and Zhang, 2004). In China, many studies have concentrated on resistance rates of Salmonella to various types of antimicrobial drugs (Yang *et al*., 2008) but few studies have examined resistance to chloramphenicol drugs. In this study, Salmonella resistance to chloramphenicol drugs was investigated and detection of pathogenic Salmonella carrying chloramphenicol resistance genes was performed to clarify the development and transmission route of drug resistance and provide an experimental basis to guide veterinary rational selection and use of chloramphenicol drugs in clinical practice to reduce the transmission of drug resistance.

MATERIALS AND METHODS

Strain collection, identification and serotyping: Salmonella samples were collected from chicken hatcheries, farms and slaughter houses located in Shandong Province and isolated in the Institute of Animal Science, Shandong Academy of Agricultural Sciences, China. Salmonella was isolated and identified as previously described by Cui *et al*. (2006). The samples were placed in sterile fluid Selenite Cysteine (SC) medium at 37°C and cultured for 24 h.

The culture solutions were then streaked onto chromogenic medium CHRO Magar Salmonella at 37°C for 24 h and the characteristics of colony morphology were observed. A single colony with neat purple edge was determined as suspected Salmonella. After the isolates were purified and cultured, the *invA* gene was detected by PCR technique (Wang *et al*., 2008). The positive isolates were identified as Salmonella strain.

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Table 1: Primer sequences of resistance gene of Salmonella

Antibiotic type	Resistance genes	Primers	Accession No.	Size (bp)
Chloramphenicols	*catA1*	F: CATTCACCCGACGCACTT R: TTATCACTTATTCAGGCGTAGCAC	VOO622	952
	catA2	F: GAACACTTTGCCCTTTATCGTC R: TCCTGCTGAAACTTTGCCATCGT	X53796	482
	catA3	F: TGZTGAGTTGAGAATGGCGATA R: GAGAGCGGCAATAACAGTCTA	XO7848	358
	cmlA	F: GCGGGCTATCTTTGCGTTTC R: AAGTAGACTGCCGTGACCGTTCC	M64556	665
	floR	F: TCCTGAACACGACGCCCGCTAT R: TCACCGCCAATGTCCCGACGAT	AJ251806	962

Using Ningbo Tianrun 200 kinds of Salmonella diagnostic serum, through Glass Agglutination Method for Salmonella serotype identification according Kauffmann-White antigen retrieval table to retrieval salmonella serotype. *Salmonella enterica* serovar pullorum strains CVCC533 and *Salmonella enterica* serovar typhimurium standard strains CVCC541 as the positive control, physiological saline as negative control.

Drug susceptibility testing: All isolates were tested by the Broth Microdilution Method based on CLSI standards for susceptibility to the following 3 antibiotics: chloramphenicol, florfenicol, thiamphenicol. *Escherichia coli* ATCC25922 was used as control strain in drug susceptibility testing and results were determined with reference to the CLSI standards (CLSI, 2007, 2008).

PCR amplification and sequence analysis: DNA template was extracted by boiling method. Primers of phenicols resistance genes were designed and synthesized with reference to the resistance gene sequences in GenBank and relevant literature (Chen *et al*., 2004; Lu *et al*., 2011). Phenicols resistance genes were amplified with primers shown in Table 1. PCR was carried out with a 20 μL reaction mixture consists of 1 μL of template DNA, 1 μL each of 10 nmol L^{-1} forward primer and reverse primer, 7 μL of double distilled water and 10 μL of 2x PCR Mix. The PCR amplification products of resistance genes were recovered and purified with gel kits and were then sequenced and analyzed by Beijing Sangon Biological Engineering Technology and Service Company Limited. In addition, analysis of genes homology was performed with corresponding resistance gene sequences in GenBank.

RESULTS AND DISCUSSION

Isolation and identification: Eighty *Salmonella enterica* serovar Indiana strains were isolated from 376 samples obtained from chicken hatcheries, farms and slaughter houses located in Shandong Province with an isolation rate of 13.88%. The isolation rate of *Salmonella enterica* serovar Indiana was highest in the slaughter houses (22.05%, 30 strains) while those in the hatcheries and farms were 20.00% (24 strains) and 21.67% (26 strains), respectively. Results are shown in Table 2.

Table 2: Percentage of Salmonella isolated from different sources

Source of strains (No.)	Serotype	No. of isolates	Separation rate (%)
Hatchery (120)	*Salmonella enterica* serovar Indiana	24	20.00
Farms (120)	*Salmonella enterica* serovar Indiana	26	21.67
Slaughter house (136)	*Salmonella enterica* serovar Indiana	30	22.05

Drug susceptibility testing: The resistance rate of the isolates to florfenicol was 95% to chloramphenicol was 76.25% and to thiamphenicol was 71.25% (Table 3). Additionally, marked differences were observed in the resistance rates of *Salmonella enterica* serovar Indiana isolated from the different chicken facilities with resistance rates to chloramphenicol of 50.00, 83.33 and 93.30% to florfenicol of 83.33, 100 and 100% and to thiamphenicol of 63, 65 and 77% in the hatcheries, farms and slaughterhouses, respectively. Results are shown in Fig. 1.

Detection of drug resistance genes: The *catA1* gene was identified in 54 of the 80 strains found to be resistant to chloramphenicol and the *floR* gene was identified in 74 of 80 strains and the *cmlA* gene in 5 of 80 stains with positive rates of 67.5 92.5 and 6.25%, respectively. The *catA2* and *catA3* genes were not identified. Interestingly, researchers found that 4 isolates carried *catA1*, *floR* and *cmlA* genes from the farms (2), slaughterhouses (2) and 49 isolates carried *catA1* and *floR* genes from the hatchery (4), farms (19) and slaughter houses (26). Results are shown in Table 4.

In this study, 80 strains of *Salmonella enterica* serovar Indiana were isolated from the chicken hatcheries, farms and slaughter houses located in Shandong Province which included isolates of different age in days, diversity and representativeness. In total, 150 strains of Salmonella

Table 3: MIC of 80 Salmonella isolates to the chloramphenicols

	Assessment standards			Results		
Drugs	Resistance	Intermediary	Sensitive	Resistance	Intermediary	Sensitive
Chloromycetin	≥32	≤8≥32	≤8	76.25% (60/80)	1.25% (1/80)	12.5% (1/80)
Florfenicol	≥16	≤4≥16	≤4	95.00% (76/80)	0	5.00% (4/80)
Thiamphenicol	≥32	≤8≥32	≤8	71.25% (57/80)	23.75% (19/80)	5.00% (4/80)

Table 4: Positive rates of resistance genes in resistant strains

Drugs	No. resistant strains	Resistance genes	No. positive strains	Positive rate (%)
Chloramphenicols	80	*catA1*	54	67.5
		floR	74	92.5
		cmlA	5	6.25

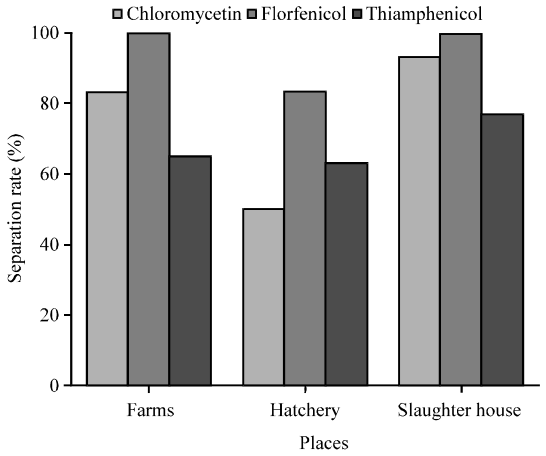

Fig. 1: The resistance rates of *Salmonella enterica* serover Indiana to chloramphenicol from different places

were isolated at an isolation rate of 39.90% which was lower than the 53.33% observed in the epidemiological analysis of chicken salmonellosis in Shanxi Province by Xue *et al.* (2010). Further, the isolation rate (21.27%) of the 80 Salmonella Indiana strains isolated in this study was higher than that reported by Wu *et al.* (2012) indicating the prevalence of *Salmonella enterica* serovar Indiana in Shandong Province.

The resistance rates of *Salmonella enterica* serovar Indiana isolated from the three different chicken facilities to florfenicol and thiamphenicol increased successively, suggesting that along with the increase in age in days and exposure to drugs, the successive rise in resistance rates was harmful to poultry farming. The highest resistance rate to chloramphenicol in the farms reached 93.33% which was related to the significant use of chloramphenicol during feeding to ensure poultry health. The resistance rates of *Salmonella enterica* serovar Indiana to chloramphenicol, florfenicol and thiamphenicol were 76.25, 95 and 71%, respectively which were considerably higher than the drug resistance rates of 30 Salmonella strains to aminoglycoside, chloramphenicol and tetracycline (56, 20 and 33.3%, respectively) observed in the drug resistance testing by Ma *et al.* (2006). Such results may be closely related to the medication used in different regions. Thus, it is important to ensure rational use of antimicrobial drugs.

Genetic mechanisms of bacterial antibiotic resistance are associated with the proteins encoded directly by resistance genes or the mutations occurring at the sites of gene action (Wei and Chen, 2006). There are two mechanisms in Salmonella resistance to chloramphenicol, the first is the transformation of chloramphenicol and thiamphenicol into metabolites without antibacterial activity by the *cat* gene regulating acetyl transferase synthesis, the second is related to active efflux in bacteria which includes the *cmlA* and *floR* genes.

In China, PCR detection of pathogenic Salmonella resistance genes to aminoglycoside, tetracycline, sulfonamide and chloramphenicol has been reported previously (Hao *et al.*, 2011; Pan *et al.*, 2002). In the study, the detection rates of drug resistance genes *floR*, *catA1* and *cmlA* were 92.5, 67.5 and 6.25%, respectively while catA2 and catA3 were not identified. Liao *et al.* (2011) detected drug resistance genes of Salmonella to chloramphenicol in 98 strains of which catA1 was identified in seven strains at a detection rate of 7.14%. These findings were significantly different from the results of the present study, suggesting that different isolated strains from different regions may result in varied prevalence and distribution of resistance genes. Drug resistance was found in a few strains in which catA2 and catA3 were not identified by detection. Although some strains showed drug resistance, the corresponding resistance genes failed to be identified. This may be related to other drug resistance genes or the existence of other mechanisms of drug resistance in the strain (Deng *et al.*, 2007).

CONCLUSION

Analysis of *Salmonella enterica* serovar Indiana resistance to chloramphenicol drugs in Shandong Province demonstrated a high detection rate of Salmonella indicating that salmonellosis was very serious problem in this area. Studies on susceptibility characteristics and genes relevant to the development of drug resistance in

Salmonella enterica serovar Indiana in Shandong Province may contribute to perform reasonable interventions in the food chain source and provide reference for practicaluse of medication in livestock. It may also assist in reducing and preventing the development of Salmonella resistance to chloramphenicol drugs and guaranteeing food safety.

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