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Characterization of Multi-Drug Resistance in Salmonella Indiana from Chicken in China

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Abstract: This research studies the characteristics of multidrug-resistant Salmonella from chicken. The Salmonella collected from parts of Shandong Province, serotype was measured by the method of Kauffmann-White, researchers using the Microdilution Method test the sensitivity of the isolates to 16 antimicrobial agents, the 12 resistance genes were detected by PCR, the relationship was analyzed between resistant phenotype and gene type. The results showed that 60 strains of Salmonella Indiana were isolated from 80 isolates of Salmonella. Drug susceptibility testing indicated that isolated bacterial strains to amoxicillin-clavulanic acid, cefazolin, polymyxin, ampicillin, doxycyclinea and trimethoprim 16 antimicrobial drugs resistance. The 88.33% isolates were resistant to 12-15 antimicrobial agents not found isolates of <3 antimicrobial agents. The 8 resistance genes were characterized by PCR that with *int1*, bla_{TEM}, *aac(6')-Ib-cr*, *floR*, *catA1*, *tetA*, *strA* and *cmlA*. More than 90% strains carrying resistance gene *int1*, bla_{TEM}, *floR* and *aac(6')-Ib-cr*. These result indicated that resistance genes were relevant to the resistant type of antimicrobial agents.

Key words: Salmonella indiana, multi-drug resistance, resistance genes, serotype, antimicrobial agents

INTRODUCTION

Salmonella is a serious zoonotic pathogen which causes infectious diarrhea in humans and is the leading cause of bacterial food poisoning outbreaks and incidence in China (Salam and Tothill, 2009). The use of antibacterial drugs plays an important role in the control and prevention of salmonellosis in humans and livestock. Therefore, pathogen resistance has become a focus of global attention and avian Salmonella resistance has also been duly noted. Previous studies have indicated that avian Salmonella resistance to antimicrobial drugs is of serious concern in many countries around the world (Witte, 1998). Zhang *et al*. (2012) reported a rate of 100% for tetracycline resistance and Parveen *et al*. (2007) found 53.4% of Salmonella strains resistant to multiple antibiotics in poultry slaughterhouses. Multidrug-resistant non-typhoid Salmonella is a critical topic in recent research. In the present study, drug susceptibility testing on Salmonella indiana isolated from different chicken hatcheries, farms and slaughterhouses in Shandong Province to 16 antimicrobial drugs commonly used in clinical practice was performed and the relationship between drug resistance phenotype and genotype was analyzed to provide a scientific basis for investigations on mechanisms of multidrug resistance in Salmonella.

MATERIALS AND METHODS

Strains and medicine: Eighty Salmonella strains were isolated from chicken samples obtained from different chicken houses located in Shandong Province. Selenite cystine enrichment broth was purchased from Qingdao Haibo Biotechnology Co., Ltd. Nutrient broth and Mueller-Hinton broth were purchased from Beijing Aoboxing Biotechnology Co., Ltd. CHROMagar™ Salmonella Chromogenic Medium was purchased from Zhengzhou Bosai Institute of Biotechnology. Ceftiofur, enrofloxacin, ciprofloxacin, chloramphenicol, polymyxin and other standard substances were purchased from China Institute of Veterinary Drug Control.

Salmonella isolation, identification and serotyping: All samples were cultured in sterile Selenite Cystine broth

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(SC) at 37°C for 24 h then were selected by chromogenic medium for Salmonella (CHROM agar, Paris, France). Only the purple-colored mono-colony on the culture plate was regarded as presumptive Salmonella colony. Picked up a purple-colored mono-colony from each plate to culture then collected DNA to test *invA* gene by PCR Method to confirm Salmonella.

Salmonella isolates were serotyped by diagnostic serum of Salmonella (Tianrun, NingBo, CN) according to the Kauffmann-White scheme. Researchers used saline as negative control and CVCC533 and CVCC541 as positive control.

Antimicrobial sensitivity: The Minimum Inhibitory Concentrations (MICs) of Salmonella isolates were determined by the broth microdilution method according to guidelines form the Clinical and Laboratory Standards Institute (CLSI, 2007, 2008). The usual clinic used antimicrobials were tested such as amoxicillin-clavulanic acid, cefazolin, ceftiofur, enrofloxacin, ciprofloxacin, nalidixic acid, chloramphenicol, polymyxin and so on. When the MIC distribution of antimicrobials was bimodal, the breakpoint was set as the midpoint between the peaks of each MIC distribution.

PCR amplification and DNA sequencing resistance genes, integrase genes: According to the report of Ng *et al.* (2001), antimicrobial resistance genes were detected. The genes and primers showed in the Table 1. The obtained DNA sequences were analyzed for sequence homology in the GenBank using the BLAST program.

Table 1: Primer sequences of resistance gene of Salmonella

Resistance genes	(5'-3') Forward primers (5'-3') Reverse primers	Accession No.	Size (bp)
bla_{TEM}	CAGCGGTAAGATCCTTGAGA ACTCCCCGTCGTGTAGATAA	AY463797	643
catA1	CATTCACCCGACGCACTTTT ATCACTTATTCAGGCGTAGCAC	V00622	952
cmlA	GCGGGCTATCTTTGCGTTTC AAGTAGACTGCCGTGACCGTTCC	M64556	665
floR	TCCTGAACACGACGCCCGCTAT TCACCGCCAATGTCCCGACGAT	AJ251806	962
StrA	CGACTTCTTACCGGACGAGGAC ACAGGTTGCGAAACGTGCCAAT	NC_009981	422
tetA	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	X75761	210
qnrA	TTCAGCAAGAGGATTTCTCA GGCAGCACTATTACTCCCAA	AY070235	500
qnrB	CCTGAGCGGCACTGAATTTAT GTTTGCTGCTCGCCAGTCGA	DQ351241	671
aac(6')-Ib-cr	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	EU543272	482

RESULTS AND DISCUSSION

Strain isolation and identification: Eighty Salmonella strains were isolated from 255 chicken samples obtained from different chicken houses located in Shandong Province with an isolation rate of 31.37% (80/255). Sixty Salmonella indiana strains were found among the 80 isolated Salmonella strains.

Drug susceptibility testing: The susceptibility testing results of 16 antimicrobial drugs are shown in Fig. 1. The isolates were generally resistant to the 16 antimicrobial drugs at resistance rates of >55% (excluding polymyxin) with a resistance rate of >95% to nalidixic acid, sulfafurazole, trimethoprim, tetracycline and doxycycline of 85% to amoxicillin-clavulanic acid, cefazolin, ceftiofur, norfloxacin, florfenicol, gentamicin and kanamycin of 60% to enrofloxacin, danofloxacin and chloramphenicol and of 5% to polymyxin, respectively.

Drug resistance phenotype: There were 32 drug resistance phenotypes in the 60 isolated Salmonella indiana strains, mainly with the resistance phenotype of m-z-c-e-o-d-n-l-f-s-tm-t-x-g-k in 14 strains, accounting for 23.33% of the total Salmonella indiana strains and secondarily with the resistance phenotypes of m-z-c-o-d-n-l-f-s-tm-t-x-g-k and m-z-c-o-n-f-s-tm-t-x-g-k in 4 strains, accounting for 6.67% of the total. Results are shown in Table 2.

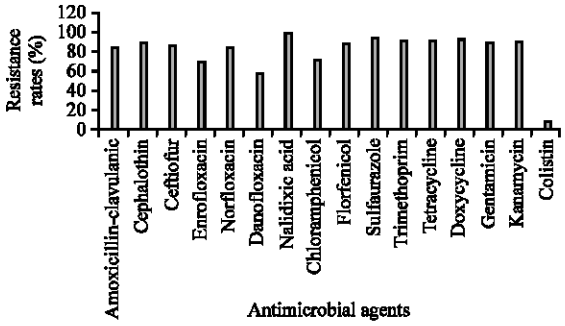

Fig. 2: The multi-resistance of 60 Salmonella isolated to 16 antimicrobial agents

Table 2: Distribution of antimicrobial resistance genes among Salmonella

Resistance genes	No. isolates with resistance genes	Resistance rate (%)
int1	48	80.00
bla_{TEM}	44	73.33
aac(6')-Ib-cr	55	91.67
floR	55	91.67
catA1	55	91.67
tetA	57	95.00
strA	57	95.00
cmlA	6	10.00

Multi-drug resistance analysis: Multidrug resistance of the 60 isolated Salmonella indiana strains to the 16 antimicrobial drugs is shown in Fig. 2 which included 9, 14, 16 and 14 strains resistant to 12, 13, 14 and 15 drugs, respectively, accounting for 88.33% of the total Salmonella strains. Seven strains were resistant to 4-11 drugs, accounting for 11.67% of the total. Strains resistant to <3 drugs were not found.

Drug resistance gene: Using ten pairs of synthetic primers, drug resistance genes were detected in the 60 Salmonella indiana strains with the results shown in Table 2. Eight resistance genes *int1*, bla_{TEM}, *aac (6')-Ib-cr*, *floR*, *catA1*, *tetA*, *strA* and *cmlA* were found widely in the strains, among which >90% (55 strains) carried int1, bla_{TEM}, aac (6')-Ib-cr, floR, catA1, tetA and strA and 10% (six strains) carried cmlA without the detection of qnrA and qnrB. Sequencing results showed that these drug resistance genes were highly homologous with the corresponding gene sequences in the GenBank database. >95% of the 60 Salmonella indiana strains carried the drug resistance genes bla_{TEM}, *floR* and *aac (6')-Ib-cr* which were the commonest resistance gene combinations detected in this study. The results of drug susceptibility testing, resistance phenotype and multidrug resistance analysis were compared and showed that the drug resistance phenotypes and genotypes accorded at high rates as shown in Table 3.

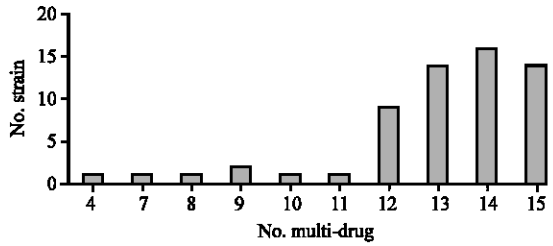

Fig. 2: The multi-resistance of 60 Salmonella isolated to 16 antimicrobial agents

In the past 20 years, the increasing isolation rate of multidrug-resistant Salmonella in various serotypes has been reported worldwide (Guerra *et al*., 2001). In this study, 80 Salmonella strains were isolated from 255 chicken samples obtained from different chicken houses located in Shandong Province with an isolation rate of 31.37% (80/255) which was significantly higher than the 10.09% reported by Li *et al*. (2008). By serological identification a Salmonella indiana isolation rate of 75% (60 strains) was found among the 80 Salmonella strains which was considerably higher than the 12% in Shaanxi Province reported by Yang *et al*. (2010) indicating that Salmonella indiana was a seriously prevalent Salmonella serotype in Shandong Province drug susceptibility testing results showed that the 60 Salmonella indiana isolates were generally resistant to the 16 antimicrobial drugs such as amoxicillin-clavulanic acid, cefazolin, nalidixic acid, chloramphenicol, florfenicol, sulfafurazole, trimethoprim, tetracycline, gentamicin and polymyxin at resistance rates of >55% (excluding polymyxin) which were similar to those reported by De Oliveira *et al*. (2005)

Table 3: Analysis of resistance phenotype and resistance genes phenotype of 60 Salmonella

No. multi-drugs	Resistance phenotype (No.)	Resistance genes phenotype
4	n-l-s-tm (1)	bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*
7	m-o-n-s-tm-t-x (1)	*int1*-bla_{TEM}
8	d-n-l-f-s-tm-t-x (1)	*aac(6')-Ib-cr-floR-catA1-tetA-strA*
9	m-e-o-d-n-s-tm-t-x (1)	*int1*-bla_{TEM}-*qnrS-tetA-strA*
	m-z-e-o-n-l-tm-t-x (1)	*int1*-bla_{TEM}-*qnrS*
10	m-z-e-o-n-s-tm-t-x-k (1)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*
11	z-c-o-n-f-s-tm-t-x-g-k (1)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*
12	z-c-o-n-l-f-s-tm-t-x-g-k (1)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*
	m-e-d-n-l-f-s-tm-t-x-k-b (1)	*aac(6')-Ib-cr-floR-catA1-tetA-strA*
	m-z-c-e-o-n-l-f-s-tm-g-k (1)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-cmlA-tetA-strA*
	m-z-c-o-n-f-s-tm-t-x-g-k (4)	*int1*-bla_{TEM}-*qepA-aac(6)-Ib-cr-floR-catA1-tetA-strA*
13	m-z-c-e-o-n-f-s-tm-t-x-g-k (2)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*
	m-z-c-o-d-n-f-s-tm-t-x-g-k (2)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*
	z-c-e-o-n-l-f-s-tm-t-x-g-k (2)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*
	m-z-c-o-n-l-f-s-tm-t-x-g-k (3)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*
	m-z-c-e-o-n-f-s-t-x-g-k-b (1)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*
14	m-z-c-o-d-n-l-f-s-tm-t-x-g-k (4)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-cmlA-tetA-strA*
	z-c-e-o-d-n-l-f-s-tm-t-x-g-k (1)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*
	m-z-c-e-o-n-l-f-s-tm-t-x-g-k (4)	*int1-aac(6')-Ib-cr-floR-catA1-tetA-strA*
	m-z-c-e-d-n-l-f-s-tm-t-x-g-k (2)	*aac(6')-Ib-cr-floR-catA1-tetA-strA*
	m-z-c-e-o-d-l-f-s-tm-t-x-g-k (1)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-cmlA-tetA-strA*
15	m-z-c-e-o-d-n-l-f-s-tm-t-x-g-k (14)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-cmlA-tetA-strA*
	m-z-c-e-o-d-n-f-s-tm-t-x-g-k-b (1)	*int1*-bla_{TEM}-*aac(6')-Ib-cr-floR-catA1-tetA-strA*

m-(amoxicillin-clavulanic acid), z-(cephalothin), c-(ceftiofur), e-(enrofloxacin), o-(norfloxacin), d-(danofloxacin), n-(nalidixic acid), l-(chloramphenicol), f-(florfenicol), s-(sulfafurazole), tm-(trimethoprim), t-(tetracycline), x-(doxycycline), g-(gentamicin), k-(kanamycin), b-(colistin)

and higher than those in 25 Salmonella strains reported by Zhang *et al.* (2007) and those observed by Hsu *et al.* (2006). This may be closely related to the strain source, drug use and feeding management. With a relatively low resistance rate of 5%, polymyxin may be used to control Salmonella currently but its use must be judicious and practical. In terms of multidrug resistance there were 9, 14, 16 and 14 Salmonella strains resistant to 12, 13, 14 and 15 drugs, respectively accounting for 88.33% of the total strains. Seven strains were resistant to 4-11 drugs, accounting for 11.67% of the total. Serious multidrug resistance was found in the 60 Salmonella indiana strains obtained from chickens which was consistent with that reported by Pan *et al.* (2002), Biendo *et al.* (2005) found a multidrug resistance rate of 98% in 51 *Salmonella typhimurium* strains from humans and (Graziani *et al.*, 2008) showed that 64% of Salmonella strains were resistant to >4 drugs which again clearly reflects serious multidrug resistance in Salmonella. This resistance is associated with the use of antimicrobial drugs. Therefore in clinical practice, antimicrobial drugs should be used rationally with various types of medication to relieve the selection pressure for bacteria and reduce the probability of bacterial resistance (Ma *et al.*, 2006).

In this study, ten pairs of primers were designed and PCR detection of common resistance genes to the 16 antimicrobial drugs such as tetracyclines, aminoglycosides, β-lactams, chloramphenicols and quinolones was performed in the 60 Salmonella indiana strains. Results showed that eight resistance genes *int1*, bla_{TEM}, *aac (6')-Ib-cr*, *floR*, *catA1*, *tetA*, *strA* and *cmlA* were prevalent in the strains while qnrA and qnrB were not detected in any of the isolates. Multiple drug resistance genes were found in >90% of the strains, revealing the complexity of the current strains carrying drug resistance genes in clinical practice.

Analysis of the drug resistance phenotypes and genotypes in Salmonella showed that the resistance genes accorded with their resistance phenotypes at high rates in most of the strains. However, a correlation between resistance gene and phenotype was not found in all strains. For example in a few strains resistant to β-lactams and sulfonamides, the corresponding resistance genes were not amplified which may be related to the existence of other resistance mechanisms or resistance genes that were not detected. In this study, researchers found some strains with resistance genes did not show drug resistance. For example, although the *cat*A1 gene mediating chloramphenicol resistance was detected in some strains those strains did not show drug resistance. This may be because the resistance gene was affected by some factors during the expression process and did not show the resistance phenotype. This requires further investigation.

CONCLUSION

The results of drug susceptibility testing and resistance gene detection showed a serious prevalence of multidrug resistance in Salmonella from farmed chickens. Therefore, improvements in Salmonella resistance monitoring are required to provide a scientific basis for rational selection and use of antimicrobial drugs in clinical practice and reduce Salmonella resistance.

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REFERENCES

Biendo, M., G. Laurans, D. Thomas, D. Thomas, B. Canarelli and F. Hamdad-Daoudi *et al.*, 2005. Molecular characterisation and mechanisms of resistance of multidrug-resistant human Salmonella enterica serovar typhimurium isolated in Amiens (France). Int. J. Antimicrob. Agents, 26: 219-229.

CLSI, 2007. Performance standards for antimicrobial susceptibility testing. 17th Information Supplement, M100-S17, Vol. 27, No. 1, Clinical and Laboratory Standards Institute, Wayne, PA., USA.

CLSI, 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard-third edition. Informational Supplement, CLSI Document M31-A3, Vol. 28, No. 2, Clinical and Laboratory Standards Institute, Wayne, PA., USA.

De Oliveira, D.S., F.S. Flores, L.R. dos Santos and A. Brandelli, 2005. Antimicrobial resistance in *Salmonella enteritidis* strains isolated from broiler carcasses, food, human and poultry-related samples. Int. J. Food Microbiol., 97: 297-305.

Graziani, C., L. Busani, A.M. Dionisi, C. Lucarelli and S. Owczarek *et al*., 2008. Antimicrobial resistance in *Salmonella enterica* serovar typhimurium from human and animal sources in Italy. J. Vet. Microbiol., 128: 414-418.

Guerra, B., S.M. Soto, J.M. Arguelles and M.C. Mendoza, 2001. Multidrug resistance is mediated by large plasmids carrying a class 1 integron in the emergent *Salmonella enteric* serotype. Antimicrob. Agents. Chemother., 45: 1305-1308.

Hsu, S.C., T.H. Chiu, J.C. Pang, C.H. Hsuan-Yuan, G.N. Chang and H.Y. Tsen, 2006. Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. Int. J. Antimicrob. Agents, 27: 383-391.

Li, S., F. Yang and X. Liu, 2008. Investigation of chicken pathogenic bacteria and drug resistance tendency scale in Northern Henan. Poult. Husbandry Dis. Control, 9: 7-9.

Ma, M.G., H.N. Wang, Y. Yu, C.Z. Li, D. Zhang, Y.F. Yang and S.G. Liu, 2006. Detection of antimicrobial resistance genes of pathogenic Salmonella from swine. Acta Veterinaria Zootechnica Sinica, 37: 65-70.

Ng, L.K., I. Martin, M. Alfa and M. Mulvey, 2001. Multiplex PCR for the detection of tetracycline resistant genes. Mol. Cell. Probes, 15: 209-215.

Pan, Z.M., X.A. Jiao, W.B. Liu, S. Gao and Z.Y. Ni *et al*., 2002. The surveillance for the antimicrobial resistance of Salmonella pullorum. Acta Veterinaria Zootechnica Sinica, 33: 377-383.

Parveen, S., M. Taabodi, J.G. Schwarz, T.P. Oscar, J. Harter-Dennis and D.G. White, 2007. Prevalence and antimicrobial resistance of Salmonella recovered from processed poultry. J. Food Prot., 70: 2466-2472.

Salam, F. and I.E. Tothill, 2009. Detection of *Salmonella typhimurium* using an electrochemical immunosensor. Biosensors Bioelectron., 24: 2630-2636.

Witte, W., 1998. Medical consequences of antibiotic use in agriculture. Science, 279: 996-997.

Yang, B., X. Zhang, D. Qu, M. Xi and S. Cui *et al*., 2010. Serotypic and genotypic characterization of Salmonella serovars from retails meat in Shaanxi province (2007-2008). Acta Microbiologica Sinica, 50: 654-660.

Zhang, C., Y. Zhao, Q. Zhou, F. Liu and C. Li *et al*., 2012. Identification and resistance of pathogenic *Salmonella* isolated from chickens. Chin. J. Vet. Drug, 46: 11-13.

Zhang, X.Y., F.H. Zhao, L. Song, H. Zhao, X. Lu, Y. Ning and G. Gao, 2007. Study on serotype identification and susceptibility for *Avian salmonella*. China Poult., 29: 18-36.