

Preplanned Studies

Prevalence of *Salmonella* and Antimicrobial Resistance in Isolates from Food Animals — Six PLADs, China, 2019

Tingting Cao¹; Peng Liu¹; Yiming Li¹; Mingquan Cui²; Chunping Zhang²; Yang Wang¹; Zhangqi Shen¹; Jianzhong Shen¹; Yuebin Ke³; Shaolin Wang^{1,†}; Yongning Wu^{4,‡}

Summary

What is already known about this topic?

Salmonella causes acute and chronic diseases in food animals, and infected food animals are one of the most important source of human infection.

What does this report contribute?

The prevalence of *Salmonella* was 10.5% in chicken samples, 24.4% in pig, 23.3% in duck, and 29.4% in milk. *Salmonella* isolates were highly resistant to ampicillin (59.60%).

What are the implications for public health practices?

Data on *Salmonella* infections among food animals in China could help identify sources and factors related to the spread of *Salmonella* in food animals and food production chains.

Salmonella bacterial infections have become a major public health issue, causing a wide range of clinical manifestations, including acute gastroenteritis and bacteremia. Antibiotics are commonly used to treat and control salmonellosis in food animals, contributing to the increasing prevalence of antibiotic-resistant *Salmonella* that has been attracting worldwide attention (1). Thus, investigating the prevalence of resistance-related genes in *Salmonella* could enhance the understanding of drug impacts on epidemiology. The study mainly followed the 2019 National Surveillance Program of Antibiotic Resistance in Bacteria of Food Animal Origins to conduct animal-food sampling in 6 provincial-level administrative divisions (PLADs) in China: Hebei, Shanxi, Shandong, Sichuan, Inner Mongolia, and Beijing. In addition to a minor decline in prevalence of *Salmonella* in chicken, results showed an increase in prevalence of *Salmonella* in pigs, ducks, and milk. *Salmonella* contamination of food animals has become a serious public health threat in China. Through antimicrobial susceptibility testing, *Salmonella* of animal origin was found to have multiple drug resistance and a high rate to ampicillin (59.6%).

Therefore, in the animal breeding environment, public health practitioners should pay attention to the disinfection of the breeding farm environment and reduce the overuse of therapeutic drugs, promote the scientific use of drugs in the breeding process, and ensure the safety of public health.

In this study, a total of 1,493 non-duplicate samples were collected and stored in ESwabs (a swab-based collection kit) from animal farms and 85 from milk storage tanks on dairy farms. The sample collection method strictly complies with 2019 National Surveillance Program of Antibiotic Resistance in Bacteria of Food Animal Origins. *Salmonella* isolates were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). *Salmonella* serotyping was performed using the White-Kauffmann-Le Minor scheme. Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method according to the recommendations in the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015: M100-S25). Whole genome sequencing was conducted using an Illumina HiSeq2500 platform (Bionova Biotech Co. Beijing, China). Multilocus sequence typing (MLST) results were analyzed using MLST Version 2 (Seemann T, mlst Github <http://github.com/tseemann/mlst>), and plasmid replicon typing was conducted using online tools (Center for Genomic Epidemiology, Technical University of Denmark, <http://www.genomicepidemiology.org/>). Resistance genes were identified using SRST2 Toolkit (version 0.2.0, The University of Melbourne, <http://katholt.github.io/srst2/>).

A total of 198 *Salmonella* isolates (198/1,578, 12.6%) were obtained from food animal samples from Hebei, Shanxi, Shandong, Sichuan, Inner Mongolia, and Beijing in 2019. Samples were collected from the feces, cecum, and milk of food animals. The highest rates of *Salmonella* isolates were obtained with the samples from cows. Beijing displayed the highest *Salmonella* isolation rate among the examined PLADs (23.3%), while Hebei displayed the lowest isolation

rate (8.8%) (Table 1).

Salmonella serotyping divided 133 isolates into 35 serotypes, with 65 isolates being incapable of being typed (Table 2). *Salmonella* Enteritidis (*S. Enteritidis*; 37.6%, 50/133) was the predominant species, followed by *S. Tympnamum* (9.0%, 12/133) and *S. Kentucky* (9.0%, 12/133). Notably, multidrug resistant (MDR) strains were widely distributed among the various *Salmonella* serotypes. Among all the serotypes, *S. Agona* (77.8%) showed the highest rates of antimicrobial resistance and MDR in the present study.

The MDR rates among *Salmonella* from animal sources were different in different PLADs. Beijing displayed the highest rate of MDR, reaching 100% (Figure 1A), followed by Shandong (52.0%), Inner Mongolia (50.0%), Sichuan (48.0%), Shanxi (45.7%), and Hebei (25.0%). *Salmonella* isolates from six different PLADs were highly resistant to ampicillin (Figure 1B and 1C). A total of 18 strains of *Salmonella* with unique drug-resistant phenotypes were selected for whole-genome sequencing. Most *Salmonella* strains were resistant to multiple drugs. These strains were derived from chickens and pigs, and the MLST type was mainly ST11. The predominant serotype among these 18 *Salmonella* strains was enteritidis.

DISCUSSION

In the present study, the prevalence of *Salmonella*

was 10.5% in chicken samples, 24.4% in pig, 23.3% in duck, and 29.4% in milk. The high contamination rate of *Salmonella* in milk samples indicated that milk is an important medium for *Salmonella* transmission. These results indicated that *Salmonella* contamination of food animals in China was a serious public health problem. Better measures should thus be taken to control *Salmonella* on dairy farms. Antimicrobial susceptibility tests in this study revealed that all the 198 *Salmonella* isolates were highly resistant to at least one tested antibiotic class (penicillin, folate pathway antagonists, tetracyclines, quinolones, and fluoroquinolones). The highest rates of antimicrobial resistance were observed for ampicillin (59.6%). High prevalence of resistance to ampicillin is due to this antibiotic has been widely used in animal husbandry in China (2). At present, clinics have reported high resistance of *Salmonella* to ampicillin (53%) (3) and high resistance in food animals (68.7%) in China (4). We should carry out regular disinfection and sterilization of the breeding environment and avoid eating meat, eggs, and other dairy products that have not been treated with high temperatures. This research provided important guidance and reference value for animal breeding drugs, and provided data reference value for the detection, diagnosis, and treatment of *Salmonella* infection in clinic.

Compared with other research reports in China, the prevalence of *Salmonella* was 43.3% for chickens (5), 17.4% for pigs (6), 2.1% for ducks (7), and 1.3% for

TABLE 1. Characteristics and prevalence of *Salmonella* isolates from the 6 PLADs, China, 2019.

PLAD	Sample	Source	No. of samples	No. of isolates	Isolating rate (%)	95% CI
Shanxi	Cow	Milk	85	25	29.4	(20.0, 40.3)
	Chicken	Cecum	121	10	8.3	(4.0, 14.7)
Sichuan	Chicken	Cecum	144	14	9.7	(5.4, 15.8)
		Fecal	60	14	23.3	(13.4, 36.0)
	Pig	Fecal	90	22	24.4	(16.0, 34.6)
Beijing	Duck	Fecal	30	7	23.3	(9.9, 42.3)
Shandong	Chicken	Cecum	247	26	10.5	(7.0, 15.0)
		Fecal	712	72	10.1	(8.0, 12.6)
Inner Mongolia	Chicken	Cecum	39	4	10.3	(2.9, 24.2)
Hebei	Chicken	Fecal	50	4	8.0	(2.2, 19.2)
Total	Chicken	Cecum/fecal	1,373	144	10.5	(8.9, 12.2)
		Pig	Fecal	90	22	24.4
	Duck	Fecal	30	7	23.3	(9.9, 42.3)
	Cow	Milk	85	25	29.4	(20.0, 40.3)

Note: The six PLADs includes Hebei, Shanxi, Shandong, Sichuan, Inner Mongolia, and Beijing. The primary objective of the present study was to investigate the Isolating Rate and corresponding 95% CIs of prevalence of *Salmonella* isolates from the 6 PLADs, China, in 2019. Abbreviations: PLADs=provincial-level administrative divisions; CI=confidence interval.

TABLE 2. *Salmonella* serovar isolates obtained from chicken, pig, cow, and duck samples from the six PLADs, China, 2019.

Serovar	Chicken	Pig	Cow	Duck	Total
Enteritidis	44	5		1	50
Tymphimurium	1	6	5		12
Kentucky	12				12
Agona	4	2	3		9
Djugu	6				6
Corvallis	4				4
Paratyphi B	4				4
Essen	2	1			3
Koenigstuhl	1			1	2
Norwich	1	1			2
Schwarzengrund		2			2
Trachau	2				2
Meleagridis			2		2
Kedougou		2			2
Kingston	1				1
Shannon	1				1
Stuttgart	1				1
Bovismorbificans	1				1
Mbandaka	1				1
Uppsala		1			1
Eschweiler	1				1
Braenderup	1				1
Derby		1			1
Havana		1			1
Anatum		1			1
Waycross		1			1
Bareilly		1			1
Azteca		1			1
Gueuletapee	1				1
Bloomsbury	1				1
Hato				1	1
Powel 2	1				1
Rissen		1			1
Menden	1				1
Nola	1				1
Total	93	27	10	3	133

Note: The six PLADs includes Hebei, Shanxi, Shandong, Sichuan, Inner Mongolia, and Beijing.

Abbreviation: PLADs=provincial-level administrative divisions.

milk (8). In addition to the reduction in the prevalence of *Salmonella* from chickens, our results showed an increase in the prevalence of *Salmonella* in pigs, ducks,

and milk. In this study, the high rate of *Salmonella* contamination in cow milk samples. Other studies have reported *Salmonella* infection in raw milk and milk-related infant foods (9) as well, which might threaten the health of babies. Although the milk sample sources used in this study were limited, the findings suggested that *Salmonella* poses a threat to the milk supply. The highest rates of antimicrobial resistance were observed for ampicillin (59.6%), in agreement with the results of many previous studies on *Salmonella* isolates from food animals (10).

This study was subject to several limitations. First, the geographically concentrated nature of the samples in the present study does not represent China as a whole. Second, the types of the samples collected from each of the six PLADs and cities were not uniform, leading to sample biases as the collected samples could not represent the overall circumstances.

This was a survey of the prevalence of *Salmonella* in samples obtained from food animals from six PLADs in China. The isolates showed high antimicrobial resistance, with resistance to ampicillin being the most common. It is worth noting that in this study, *S. Enteritidis* displayed the most prevalent drug resistance and MDR. MDR *Salmonella* isolates from humans have a common ancestor with the isolates from food animals, increasing the difficulty of curing human infections and increasing healthcare costs. A nationally coordinated intervention strategy for drug use in farmed animals is needed to limit the spread of MDR *Salmonella*. Better methods for monitoring the emergence and spread of MDR *Salmonella* would facilitate disease control and treatment. To prevent these strains from becoming a worldwide pandemic, internationally coordinated intervention strategies to limit further dissemination of MDR *Salmonella* are required.

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Corresponding authors: Shaolin Wang, shaolinwang@cau.edu.cn; Yongning Wu, wuyongning@cfsa.cn.

¹ College of Veterinary Medicine, China Agricultural University, Beijing, China; ² China Institute of Veterinary Drug Control, Beijing, China; ³ Key Laboratory of Molecular Epidemiology of Shenzhen, Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong, China; ⁴ National Health Commission Key Laboratory of Food Safety Risk Assessment, Food Safety Research Unit

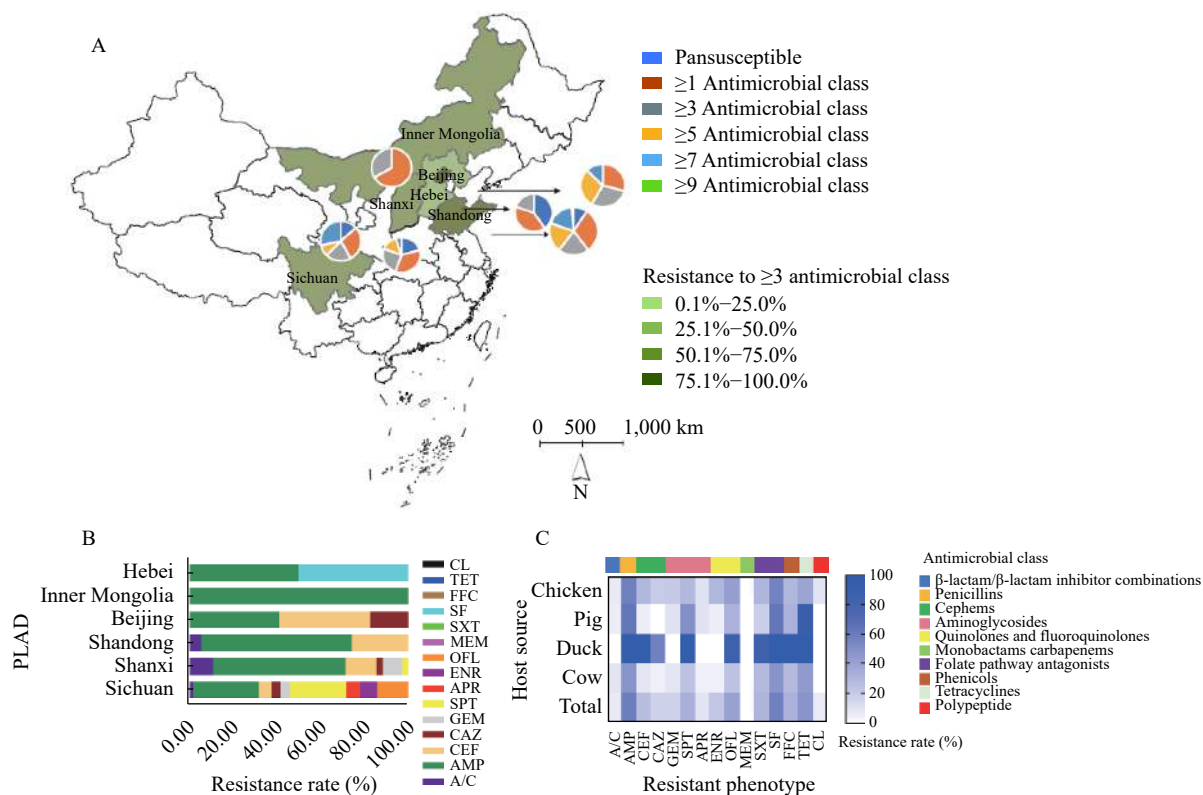


FIGURE 1. Multidrug resistance and distribution of *Salmonella* from the six PLADs, China, 2019. (A) The proportion of different antibiotic resistant types. (B) Distribution of *Salmonella* resistance phenotypes from different PLADs and cities in China. (C) Resistant phenotypes of *Salmonella* from different host sources.

Note: The six PLADs include Hebei, Shanxi, Shandong, Sichuan, Inner Mongolia, and Beijing.

Abbreviations: PLADs=provincial-level administrative divisions; A/C=amoxicillin-clavulanic acid; AMP=ampicillin; CEF=ceftiofur; CAZ=ceftazidime; GEM=gentamicin; SPT=spectinomycin; APR=apramycin; ENR=enrofloxacin; OFL=ofloxacin; MEM=meropenem; SXT=trimethoprim-sulfamethoxazole; SF=sulfamethoxazole; FFC=florfenicol; TET=tetracycline; CL=colistin.

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