Prevalence and antimicrobial resistance of *Salmonella enterica* subspecies *enterica* serovar Enteritidis isolated from broiler chickens in Shandong Province, China, 2013–2018

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ABSTRACT *Salmonella* is a major zoonotic foodborne pathogen that persists on poultry farms worldwide. The present study aimed to survey the prevalence of *Salmonella* and antimicrobial resistance of *Salmonella enterica* serovar Enteritidis (***S.* Enteritidis**) recovered from broiler chickens in Shandong Province, China. A total of 280 *Salmonella* isolates were identified from 923 broiler chicken samples between 2013 and 2018. Among the isolates, *S.* Enteritidis (n = 128, 45.7%) was the predominant serovar, and high antimicrobial resistance rates to piperacillin (**PIP**) (n = 123, 96.1%), ampicillin (**AM**) (n = 122, 95.3%), nitrofurantoin (**FT**) (n = 106, 96.1%), and tetracycline (**TE**) (n = 93, 72.7%) were observed in *S.* Enteritidis. A total of 96 (75.0%) *S.* Enteritidis isolates presented with multidrug resistance, the most frequent of which were the combination of AM, PIP, TE, and FT. Resistance to fluoroquinolone tended to increase during 2013 to 2018. Our findings provide important and updated information about the baseline antimicrobial-resistant data for food safety and a risk assessment of *S.* Enteritidis from broiler chickens in Shandong Province and will be helpful for future surveillance activities to ensure the safety of the chicken supply.

Key words: *Salmonella*, *Salmonella* Enteritidis, prevalence, broiler chicken, multidrug-resistant

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INTRODUCTION

Salmonella is a major zoonotic foodborne pathogen causing outbreaks and sporadic cases of gastroenteritis and septicemia in humans (Eng et al., 2015). Estimates suggest that 93 million enteric infections and 155,000 deaths are attributed to *Salmonella* each year (Balasubramanian et al., 2019). The contamination source of *Salmonella* is primarily food animals, particularly poultry, which is an important reservoir that can be transmitted to humans via several routes, such as consumption of contaminated poultry meat and egg products (Hald et al., 2016). It is important to examine the prevalence of *Salmonella* in food-producing animals to control foodborne salmonellosis.

The widespread and indiscriminate use of antibiotics in veterinary medicine, including food animal production, is one of the major contributors to the development and spread of antimicrobial resistance (**AMR**) (Marshall and Levy, 2011). Ampicillin (**AM**), chloramphenicol, and trimethoprim-sulfamethoxazole have been traditionally used to treat human salmonellosis. However, the emergence of drug-resistant pathogens has led to a decline in the efficacy of traditional antimicrobial therapy (Michael and Schwarz, 2016). Antibiotics commonly prescribed for these infections include fluoroquinolones (e.g., ciprofloxacin) or third-generation (extended-spectrum) cephalosporins (e.g., ceftriaxone) because of the low number of *Salmonella* isolates that are resistant to these drugs (Gilbert et al., 2016). Antimicrobial resistance may contribute to bacteremia, treatment failure, and poor clinical outcomes. *Salmonella* bacteremia is more common in drug-resistant than in susceptible infections (Crump et al.,

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2011; Krueger et al., 2014). Hence, development of resistance to these key antimicrobials is a major problem for public health.

Because of the widespread commercial trade in animal-derived food products, surveillance of the *Salmonella* serotype distribution and antibiotic resistance levels in food-producing animals from individual countries is of global importance. Shandong is the main producer of animal food in China and intensively farms chickens. However, only a few studies have investigated the prevalence and AMR in bacteria associated with broiler chickens in Shandong (Lai et al., 2014), where awareness and understanding of AMR remain limited. Therefore, in the current study, we examined the *Salmonella* serovar diversity and the prevalence of AMR in broiler chicken samples from Shandong Province, China.

MATERIALS AND METHODS

Sample Collection

A total of 923 samples were collected between January 2013 and July 2018 (Figure 1). Feces and cecal contents of broiler chickens from conventional farms were collected in 8 important farming cities in Shandong Province, including Jinan, Liaocheng, Linyi, Rizhao, Taian, Weihai, Weifang, and Yantai (Figure 2). Each sample was collected from a different animal. Fresh sterile gloves were used with every sample to avoid cross-contamination. The bag was sealed and transferred in a cooler with ice to the laboratory within 8 h.

Isolation and Identification of Salmonella

Fresh fecal samples and cecal contents were randomly collected in each farm using sterile cotton swabs that had been premoistened with buffered peptone water (Haibo, Qingdao, China). Next, the swabs of the same farm were mixed and cultured separately overnight in buffered peptone water, then inoculated into selenite cystine broth (Haibo) and tetrathionate broth (Haibo) at 37°C and 42°C for 20 h, respectively. They were ultimately streaked onto xylose lysine tergitol 4 (XLT-4; Haibo) agar plates and *Salmonella* chromogenic agar (Haibo) plates. Among suspected colonies, only 1 was picked up from a plate and checked for typical *Salmonella* colonies.

Serotyping

Salmonella spp. were serotyped using the Kauffman–White typing scheme (Grimont and Weill, 2007) by slide agglutination for detecting somatic (O) and flagellar (H) antigens with Salmonella antisera (S&A Reagents Lab Ltd., Bangkok, Thailand).

Antimicrobial Susceptibility Testing

The frozen isolates were subcultured twice, and the fresh isolates subcultured once, on tryptic soy agar plates containing 5% sheep blood (Haibo) at 37°C for 20 h. Four to 5 isolated colonies were selected from a pure culture plate to prepare bacterial suspension. Then, the antimicrobial susceptibility tests were performed using VITEK 2 AST-GN65 cards (bioM é rieux, Quebec, Canada). All results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2008; CLSI, 2013). The ATCC 25922 strain of *Escherichia coli* was used as a control. The following 17 antibiotics were tested (concentration range in µg/mL): amikacin (**AN**, 8–64), amoxicillin/clavulanic acid (**AMC**, 4/2–32/16), AM (4–32), cefalexin (**CN**, 8–64), cefovecin (**CFO**, 0.5–2), cefpodoxime (**CPD**, 0.5–4), ceftiofur (**CFT**, 1–2), chloramphenicol (**C**, 4–32), enrofloxacin (**ENR**, 0.25–4), gentamicin (**GM**, 4–32), imipenem (**IPM**, 2–16), marbofloxacin (**MRB**, 1–2), nitrofurantoin (**FT**, 16–64), piperacillin (**PIP**, 4–64), tetracycline (**TE**, 2–8), tobramycin (**TM**, 8–64), and trimethoprim/sulfamethoxazole (**SXT**, 1/19–16/304). Isolates that exhibited resistance to at least 3 classes of antimicrobial agents tested were considered multiresistant.

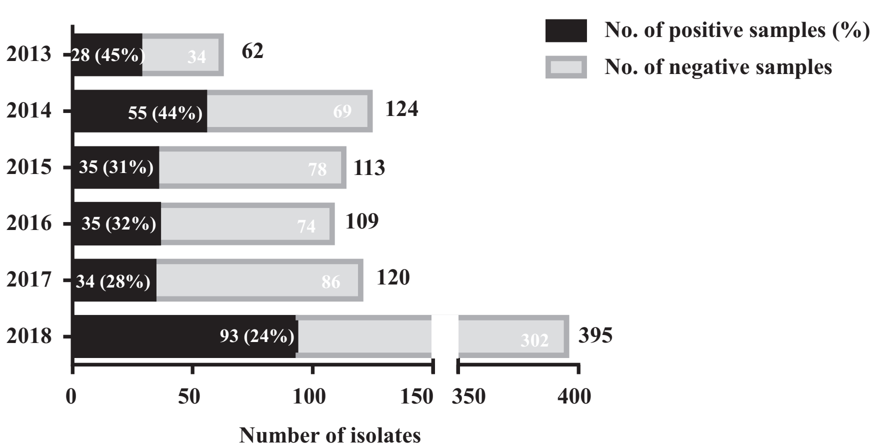

Figure 1. Comparison of the Salmonella isolation rates in broiler chickens during 2013 to 2018.

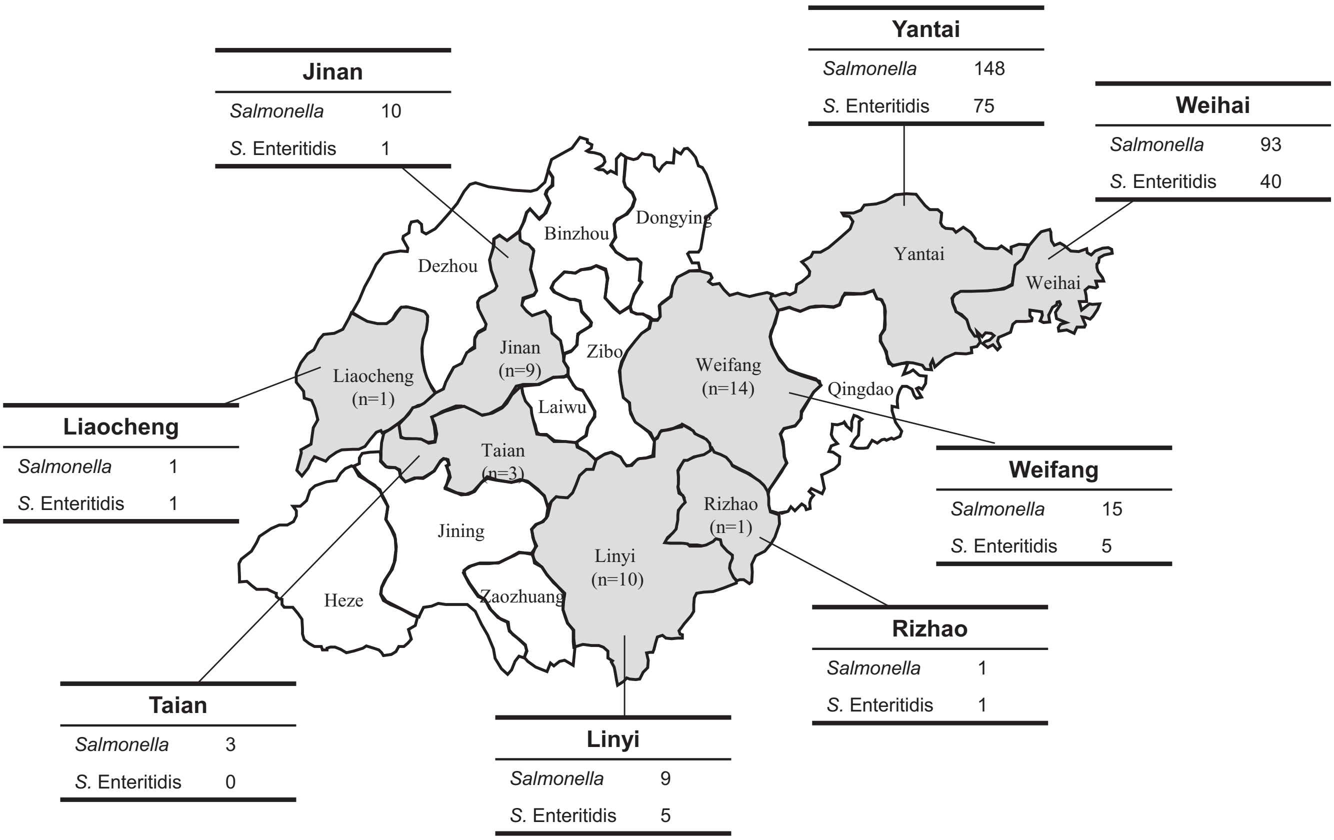

Figure 2. Sample collection regions in Shandong Province during 2013 to 2018 (n = 280).

Statistics

Statistical comparisons of the isolation rates and AMR rates among the different food-producing animals and serovars were analyzed using the chi-square test and SPSS software (SPSS Inc., Chicago, IL; version 11.5).

RESULTS

Prevalence of Salmonella in Samples Collected From Broiler Chickens

During the 6-yr study period, 280 (30.3%) *Salmonella* isolates were identified from 923 broiler chicken fecal and cecal samples. The separation proportion of *Salmonella* decreased year by year during the study, from 45% in 2013 to 24% in 2018 (Figure 1).

Salmonella Serovar *Distribution in Broiler Chickens*

Twenty-three *Salmonella* isolates were untypeable (self-agglutination). Seventeen different serovars were identified from 2013 to 2018. The most common serovar was *S.* Enteritidis (128/280, 45.7%). Other serovars included *S.* Kentucky (42/280, 15.0%), *S.* Typhimurium (27/280, 9.6%), *S.* Amager (19/280, 6.8%), *S.* Gallinae (14/280, 5.0%), *S.* Abortus equi (2/280, 0.7%), *S.* Concord (2/280, 0.7%), *S.* Blegdam (1/280, 0.4%), *S.* Dublin (1/280, 0.4%), *S.* Hissar (1/280, 0.4%), *S.* Tsevie (1/280, 0.4%), *S.* Orion (1/280, 0.4%) *S.* Okerara (1/280, 0.4%), *S.* London (1/280, 0.4%), *S.* Derby (1/280, 0.4%), and *S.* Sinstorf (1/280, 0.4%) (Table 1).

Antibiotic Resistance in Salmonella

As shown in Table 1, *S.* Enteritidis was the predominant *Salmonella* serotype (128/280, 45.7%) isolated from broiler chickens in Shandong Province during 2013 to 2018. We next explored the AMR of the *S.* Enteritidis isolates. A total of 128 *S.* Enteritidis isolates were distributed in 7 cities in Shandong Province (Figure 2). As shown in Table 2, among the 128 *S.* Enteritidis isolates, resistance to PIP, AM, FT, and TE were observed in 123 (96.1%), 122 (95.3%), 106 (82.8%), and 93 (72.7%) isolates, respectively. In comparison, resistance to other antimicrobials was less common: 31.3% (40/128) of the isolates were resistant to SXT and 10.2% (13/128) were resistant to CN. The resistance rates to ENR, CFO, GM, CFT, MRB, CPD, C, and TM were generally <10%. All isolates were susceptible to AN, AMC, and IPM.

Furthermore, the resistance rates of these *S.* Enteritidis isolates to 17 antimicrobial agents in the years 2013 to 2018 were explored. A marked increase of resistance against *S.* Enteritidis was observed for FT (from 62.5% to 88.9%) and ENR (from 0% to 13.3%). A wavy trend was observed in the resistance rates to TE and SXT of these isolates, with a peak point in 2015. In addition,

Table 1. *Salmonella* serovar distribution in broiler chickens.

Salmonella serovar (s)	No. of samples	% Of samples
Enteritidis	128	45.7%
Kentucky	42	15.0%
Typhimurium	27	9.6%
Amager	19	6.8%
Gallinae	14	5.0%
II	14	5.0%
Abortus equi	2	0.7%
Concord	2	0.7%
Blegdam	1	0.4%
Dublin	1	0.4%
Hissar	1	0.4%
Tsevie	1	0.4%
Orion	1	0.4%
Okerara	1	0.4%
London	1	0.4%
Derby	1	0.4%
Sinstorf	1	0.4%
Rough	23	8.2%
Total	280	100%

the resistance rates of *S.* Enteritidis to AN, TM, IPM, CPD, MRB, AMC, and C had been at a low level (<10%) during the study period. Conversely, these *S.* Enteritidis strains were highly resistant to AM and PIP (>87%) (Figure 3 and Table 2).

In total, 25 resistance patterns of these *S.* Enteritidis isolates to 8 categories of antimicrobials were found (Table 3). Among the 128 isolates, 3.9% (5/128) of the isolates were resistant to 5 or more categories of antimicrobials, 25.8% (33/128) of the isolates were resistant to 4 categories of antimicrobials, 45.3% (58/128) of the isolates were resistant to 3 categories of antimicrobials, and 21.1% (27/128) of isolates resistant to 2 categories of antimicrobials. A few isolates were resistant to 1 category of antimicrobial. Most *S.* Enteritidis isolates were resistant to 2 to 4 categories of antimicrobials (Figure 4). The dominant resistance pattern was AM-PIP-TE-FT (33.6%, 43/128), followed by AM-PIP-TE-SXT-FT (20.3%, 26/128), and AM-PIP-FT (14.1%, 18/128).

DISCUSSION

For the purpose of this study, we collected 280 *Salmonella* isolates from feces and cecal contents of broiler chickens in 8 cities of Shandong Province which was one of the largest producers of animal food in China for the presence of *Salmonella* and further characterized the isolates using serotyping and of antimicrobial susceptibility testing. The results showed that *Salmonella* has been isolated from all conventional farms in Shandong Province, and most of the isolates were multidrug-resistant (**MDR**) strains, suggesting that chicken farms in Shandong Province are commonly contaminated with MDR *Salmonella*, which poses a potential challenge to food safety and public health.

There had a few studies on prevalence of *Salmonella* in broiler chickens in China wherein values varied from 12.6 to 45.2% depending on the geographical (Bai et al., 2015; Kuang et al., 2015; Ma et al., 2017). Our isolation rate of 30.3% was much higher than that in Sichuan Province (12.55%) (Ma et al., 2017) and the center of China (22.0%) (Kuang et al., 2015), while was lower than that in Henan Province (45.2%) (Bai et al., 2015). In addition, the average *Salmonella*-positive rate in this study was in accordance with that in Egypt (120/420, 28.6%) (Elkenany et al., 2019) but higher than that in Wakiso District, Uganda (51/379, 13.46%) (Ball et al., 2020), Kwara State, North Central

Table 2. Antibiotic resistance rates of *S.* Enteritidis isolated from broiler chickens.

		No. (%) of antimicrobial-resistant *S.* Enteritidis isolates						
Antimicrobial category	Drugs	2013 (n = 8)	2014 (n = 42)	2015 (n = 18)	2016 (n = 19)	2017 (n = 11)	2018 (n = 30)	Total (n = 128)
Aminoglycosides	AN	0	0	0	0	0	0	0
	TM	0	0	0	0	1 (9.1)	0	1 (0.8)
	GM	0	0	1 (5.6)	4 (21.1)	1 (9.1)	0	6 (4.7)
Carbapenems	IPM	0	0	0	0	0	0	0
Nonextended spectrum cephalosporins; first and second generation cephalosporins	CN	0	3 (7.1)	1 (5.6)	2 (10.5)	4 (36.4)	3 (10)	13 (10.2)
Extended-spectrum cephalosporins; third and fourth generation cephalosporins	CFO	0	1 (2.4)	1 (5.6)	1 (5.3)	1 (9.1)	2 (6.7)	6 (4.7)
	CFT	0	1 (2.4)	1 (5.6)	1 (5.3)	1 (9.1)	1 (3.3)	5 (3.9)
	CPD	0	0	1 (5.6)	0	1 (9.1)	0	2 (1.6)
Fluoroquinolones	ENR	0	2 (4.8)	1 (5.6)	1 (5.3)	1 (9.1)	4 (13.3)	9 (7.0)
	MRB	0	1 (2.4)	1 (5.6)	1 (5.3)	1 (9.1)	1 (3.3)	5 (3.9)
Folate pathway inhibitors	SXT	2 (25)	14 (33.3)	12 (66.7)	4 (21.1)	3 (27.3)	5 (16.7)	40 (31.3)
Penicillin	AM	7 (87.5)	38 (90.5)	18 (100)	19 (100)	11 (100)	29 (96.7)	122 (95.3)
	PIP	8 (100)	38 (90.5)	18 (100)	19 (100)	11 (100)	29 (96.7)	123 (96.1)
Penicillin+β-lactamase inhibitors	AMC	0	0	0	0	0	0	0
Phenicols	C	0	0	1 (5.6)	0	0	1 (3.3)	2 (1.6)
Tetracyclines	TE	5 (62.5)	27 (64.3)	18 (100)	12 (63.2)	6 (54.5)	25 (83.3)	93 (72.7)
Nitrofurans	FT	5 (62.5)	36 (85.7)	16 (88.9)	16 (84.2)	7 (63.6)	26 (86.7)	106 (82.8)

Abbreviations: AM, Ampicillin; AMC, Amoxicillin/Clavulanic Acid; AN, Amikacin; C, Chloramphenicol; CFO, Cefovecin; CFT, Ceftiofur; CN, Cefalexin; CPD, Cefpodoxime; ENR, Enrofloxacin; FT, Nitrofurantoin; GM, Gentamicin; IPM, Imipenem; MRB, Marbofloxacin; PIP, Piperacillin; SXT, Trimethoprim/sulfamethoxazole; TE, Tetracycline; TM, Tobramycin.

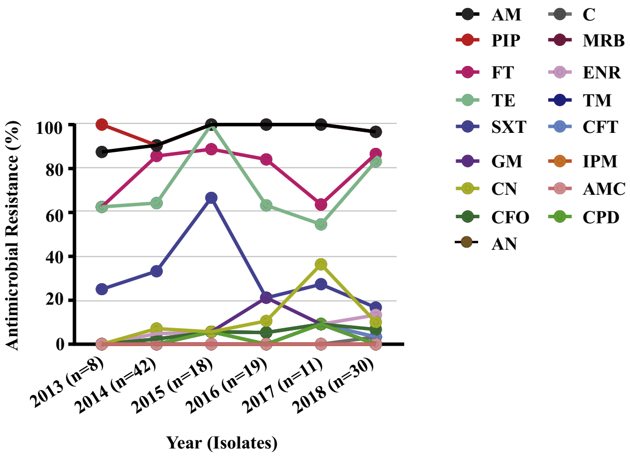

Figure 3. Antibiotic resistance rates of S. Enteritidis isolated from broiler chickens during 2013 to 2018 (n = 128). The vertical axis shows the percentage antimicrobial resistance, and the total number of strains isolated each year was set to 100. Abbreviations: AM, Ampicillin; AMC, Amoxicillin/Clavulanic Acid; AN, Amikacin; C, Chloramphenicol; CFO, Cefovecin; CFT, Ceftiofur; CN, Cefalexin; CPD, Cefpodoxime; ENR, Enrofloxacin; FT, Nitrofurantoin; GM, Gentamicin; IPM, Imipenem; MRB, Marbofloxacin; PIP, Piperacillin; SXT, Trimethoprim/sulfamethoxazole; TE, Tetracycline; TM, Tobramycin.

Nigeria (58/900, 6.4%) (Ahmed et al., 2019), northwestern Spanish (67/6577, 1.02%) (Lamas et al., 2016), and EU (1.89%) (EFSA, 2018). The differences in these isolation rates might be caused by differences in the cleaning and disinfection measures, control plans. As we know, EU had established the control program for Salmonella in poultry houses. The percent prevalence of *Salmonella* in this study highlights the potential risk to humans in Shandong Province, particularly those engaging in poultry production.

In the present study, 17 *Salmonella* serovars were identified from the samples with a notably high prevalence of *S.* Enteritidis (45.7%), followed by *S.* Kentucky (15.0%) and *S.* Typhimurium (9.6%). Among them, 2 of the serotypes (*S.* Enteritidis, *S.* Typhimurium) are involved in confirmed cases of human salmonellosis in China (Liu et al., 2018). The most common serotype identified in the present study was *S.* Enteritidis (45.7%), which was compatible with investigation results from other chicken farms in Shandong (Lai et al., 2014; Yang et al., 2019) and chicken farms in Henan and Sichuan areas of China (Li et al., 2013; Bai et al., 2015), as well as Uganda (Ball et al., 2020), EU (EFSA, 2018), and northern Poland (Witkowska et al., 2018). *S.* Enteritidis is a serovar frequently identified in many studies and is one of the most common serovars causing human salmonellosis (Pardo-Roa et al., 2019). Preventing *S.* Enteritidis dissemination in chickens is necessary to keep food safe. However, the most common isolated *Salmonella* from the chicken farms in Brazil, North Central Nigeria, and Northwestern Spanish were *S.* Minnesota, *S.* Agama, and *S.* Typhimurium, respectively (Voss-Rech et al., 2015; Lamas et al., 2016; Liu et al., 2018). The differences in these dominant serotypes might be caused by the different locations sampled. Notably, 2 strains of *S.* abortus equi, a frequently reported host-specific pathogen causing abortion in mare, were isolated from broilers samples in the study. After investigation, we found that a chicken feed additive, fish meal, was contaminated with *S.* abortus equi.

The increasing rate of AMR in *Salmonella* is a growing healthcare problem that needs to be monitored continuously. The *Salmonella* sampled in this study was highly resistant to PIP (96.1%) as well as AM (95.3%), and to a lesser extent to FT (82.8%), TE (72.7%), SXT (31.3%), and CN (10.2%). Antimicrobial susceptibility test performed on *Salmonella* in another breeder farm in Shandong showed a high resistance to streptomycin (100%), nalidixic acid (100%), AM (98.4%), and erythromycin (95.2%) (Yang et al., 2019). The high resistance to these antimicrobials observed in the present study was probably because of the use of antibiotic agents, which are incorporated into animal feed and are present at therapeutic or sub-therapeutic levels to prevent bacteriosis or promote animal growth. Almost all isolated *Salmonella* strains exhibited resistance to PIP and AM, indicating the limited therapeutic value of penicillin drugs in poultry. Notably, the AMR trend of TE and SXT increased from 2013 to 2015, whereas it decreased sharply in 2016, which may have been because of the National Action Plan to Contain Antimicrobial Resistance (2016–2020) presented in 2016 by the Chinese government (Qiao et al., 2018).

In our research, the resistance rate to cephalosporins, such as CFO and CFT, increased from 0% in 2013 to 9.1% in 2017. Lai et al. reported that the resistance rate to CFO increased from 6.1% in 2009 to 37% in 2012 in Shandong Province (Lai et al., 2014), suggesting that the resistance rate of *Salmonella* to cephalosporins has been on the rise for years. The CFT is a third-generation cephalosporin approved for use in livestock and poultry agriculture in China and has the potential to be selected for resistance to third-generation cephalosporins (Bai et al., 2015). Cephalosporins, such as ceftriaxone, are the main antimicrobials used to treat serious *Salmonella* infections in humans. Owing to similar or identical resistance mechanisms, cross-resistance between cephalosporins is common. Thus, the increased rate of cephalosporin-resistant *Salmonella* of animal origin has important public health implications. Although the resistance rate to cephalosporins was <10% in the present study, the trend in the drug resistance rate to cephalosporins should be a concern because of the potential for developing resistance directly through interference with treatment or indirectly through dissemination of resistance elements to other pathogens.

Fluoroquinolones, TE and sulfamides are the most common antimicrobials used in chicken flocks in China. An increasing resistance rate to ENR (from 0% in 2013 to 13.3% in 2018) was observed during the study period. The increasing resistance rate to ENR in this study is worrisome because fluoroquinolones have been used strategically to treat salmonellosis. This increased resistance rate may have occurred because of the indiscriminate use of

Table 3. Antimicrobial resistance patterns in *S.* Enteritidis isolates from broiler chickens during 2013 to 2018.

No. of antimicrobial resistant categories	Antimicrobial resistance categories	Antimicrobial resistance pattern	Prevalence, n (%)	
1	Penicillin	PIP	1 (0.8)	
		AM-PIP	1 (0.8)	
	Nitrofurans	FT	3 (2.3)	5 (3.9)
2	Nonextended spectrum cephalosporins + Nitrofurans	CN-FT	1 (0.8)	
	Folate pathway inhibitors + Nitrofurans	SXT-FT	1 (0.8)	
	Penicillin + Nitrofurans	AM-PIP-FT	18 (14.1)	
	Penicillin + Tetracyclines	AM-PIP-TE	7 (5.5)	27 (21.1)
3	Penicillin + Aminoglycosides + Nitrofurans	AM-PIP-GM-FT	3 (2.3)	
	Penicillin + Nonextended spectrum cephalosporins + Tetracyclines	AM-PIP-CN-TE	1 (0.8)	
	Penicillin + Nonextended spectrum cephalosporins + Fluoroquinolones	AM-PIP-CN-ENR	1 (0.8)	
	Penicillin + Fluoroquinolones + Nitrofurans	AM-PIP-ENR-FT	1 (0.8)	
	Penicillin + Nonextended spectrum cephalosporins + Nitrofurans	AM-PIP-CN-FT	2 (1.6)	
	Penicillin + Folate pathway inhibitors + Tetracyclines	AM-PIP-SXT-TE	7 (5.5)	
	Penicillin + Tetracyclines + Nitrofurans	AM-PIP-TE-FT	43 (33.6)	58 (45.3)
4	Penicillin + Nonextended spectrum cephalosporins + Tetracyclines + Nitrofurans	AM-PIP-CN-TE-FT	2 (1.6)	
	Penicillin + Nonextended spectrum cephalosporins + Aminoglycosides + Nitrofurans	AM-PIP-CN-GM-FT	1 (0.8)	
	Penicillin + Nonextended spectrum cephalosporins + Folate pathway inhibitors + Nitrofurans	AM-PIP-CN-SXT-FT	1 (0.8)	
	Penicillin + Fluoroquinolones + Tetracyclines + Nitrofurans	AM-PIP-ENR-TE-FT	2 (1.6)	
	Penicillin + Tetracyclines + Folate pathway inhibitors + Nitrofurans	AM-PIP-TE-SXT-FT	26 (20.3)	
	Penicillin + Nonextended spectrum cephalosporins + Extended-spectrum cephalosporins + Fluoroquinolones	AM-PIP-CN-CFO-CFT-ENR-MRB	1 (0.8)	33 (25.8)
5	Penicillin + Extended-spectrum cephalosporins + Phenicols + Tetracyclines + Nitrofurans	AM-PIP-CFO-C-TE-FT	1 (0.8)	
	Penicillin + Nonextended spectrum cephalosporins + Extended-spectrum cephalosporins + Fluoroquinolones + Tetracyclines	AM-PIP-CN-CFO-CFT-ENR-MRB-TE	1 (0.8)	2 (1.6)
7	Penicillin + Nonextended spectrum cephalosporins + Extended-spectrum cephalosporins + Aminoglycosides + Fluoroquinolones + Tetracyclines + Folate pathway inhibitors	AM-PIP-CN-CPD-CFO-CFT-TM-GM-ENR-MRB-TE-SXT	1 (0.8)	
	Penicillin + Nonextended spectrum cephalosporins + Extended-spectrum cephalosporins + Fluoroquinolones + Tetracyclines + Folate pathway inhibitors + Nitrofurans	AM-PIP-CN-CFO-CFT-ENR-MRB-TE-SXT-FT	1 (0.8)	2 (1.6)
8	Penicillin + Nonextended spectrum cephalosporins + Extended-spectrum cephalosporins + Aminoglycosides + Fluoroquinolones + Phenicols + Tetracyclines + Folate pathway inhibitors	AM-PIP-CN-CPD-CFO-CFT-GM-ENR-MRB-C-TE-SXT	1 (0.8)	1 (0.8)

Abbreviations: AM, Ampicillin; AMC, Amoxicillin/Clavulanic Acid; AN, Amikacin; C, Chloramphenicol; CFO, Cefovecin; CFT, Ceftiofur; CN, Cefalexin; CPD, Cefpodoxime; ENR, Enrofloxacin; FT, Nitrofurantoin; GM, Gentamicin; IPM, Imipenem; MRB, Marbofloxacin; PIP, Piperacillin; SXT, Trimethoprim/sulfamethoxazole; TE, Tetracycline; TM, Tobramycin.

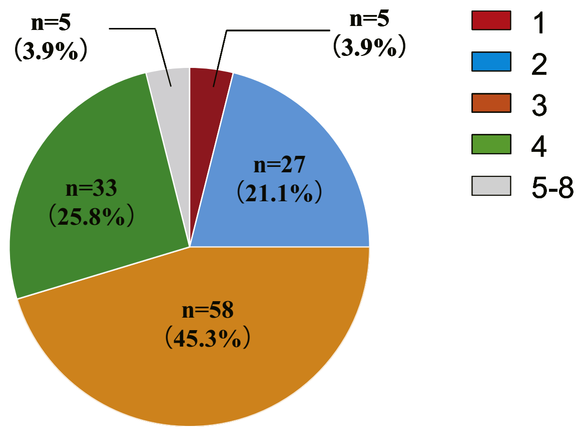

Figure 4. Number of *S.* Enteritidis isolates resistant to the indicated number of antimicrobial categories.

antimicrobials at recommended doses or at subtherapeutic doses in feed as growth promoters and as chemotherapeutic agents to control epizootics on farms. The resistance rate to ENR was lower than that observed by Yang et al. in Shandong Province (Yang et al., 2019). The differences might be because of different dosages and types of antimicrobials used in different areas and at different farms.

All *S.* Enteritidis isolates in the current study were resistant to at least 1 category of antimicrobial, which was in accordance with the study by Lai et al. in Shandong Province (Lai et al., 2014). Furthermore, we found that 75.0% of the isolates were MDR strains, which was higher than that in poultry farms of Henan (46.0%) province in China (Bai et al., 2015). These MDR strains are supported by previous observations from chicken isolates (Firoozeh et al., 2012). The MDR phenotypes of Salmonellae are clinically important, because β-lactams, TE, SXT, and quinolones are essential to treat salmonellosis in poultry (Elkenany et al., 2018). Based on the results, trials to prevent outbreaks of MDR *Salmonella* in Shandong Province are needed through functional surveillance of AMR and appropriate effective measures directed toward unregulated use of antibiotics. Probiotics are probably used as promising alternatives to antibiotics in the control of animal infections.

In summary, this study has shown that *S.* Enteritidis, *S.* Kentucky, and *S.* Typhimurium were the 3 main serotypes in the broiler chickens in Shandong Province. All *S.* Enteritidis isolates were resistant to at least one of the evaluated antimicrobials, mainly PIP, AM, FT, and TE. All isolates were susceptible to AN, IPM, and AMC. A total of 96 (75.0%) *S.* Enteritidis isolates presented MDR. Resistance to fluoroquinolone trended upward during 2013 to 2018. The overall findings suggest a high potential for transmission of the serovar Enteritidis between humans and chickens, supporting significant risks to public health posed by serovar Enteritidis infections in chickens.

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DISCLOSURES

The authors declare no conflicts of interest.

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