Original Article

Antimicrobial resistance, class 1 integrons, and horizontal transfer in *Salmonella* isolated from retail food in Henan, China

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Abstract

Introduction: Salmonellosis remains one of the most frequently occurring foodborne diseases worldwide, especially in developing countries. The increasing prevalence of multidrug resistance among *Salmonella* isolates from food has been an emerging problem in China.

Methodology: In this study, a total of 638 food samples including raw meat, seafood, vegetables, and cooked meat were collected in Henan province of China between July 2007 and August 2008 to determine the prevalence of *Salmonella*. These isolates were subjected to serotyping, antimicrobial susceptibility, presence of class 1 integrons, and horizontal transfer of integrons.

Results: The overall percentage of *Salmonella* prevalence was 9.7% (n = 62). Among these isolates, *S*. Anatum and *S*. Senftenberg were most common, and high rates of antimicrobial resistance were observed to sulfamethoxazole (90.3%), trimethoprim/sulfamethoxazole (87.1%), streptomycin (29.0%), and ciprofloxacin (25.8%). Class 1 integrons were detected in 16.1% of these isolates, and contained gene cassettes *dfrA12-aadA2*, *dfrA1-aadA1*, and *dfrA1*. Three *Salmonella* isolates could transfer their integrons and resistance genes to *Escherichia coli* by conjugation.

Conclusions: Our findings indicate that the mobile DNA elements could play an important role in the dissemination of resistance determinants among those *Salmonella* isolates.

Key words: antimicrobial resistance; *Salmonella*; retail food; integron

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Introduction

Salmonella, including more than 2,500 different serovars, represents a major cause of foodborne diseases throughout the world [1,2]. In most cases, the infections are associated with ingestion of contaminated food products, particularly those of animal origin such as poultry, eggs, beef, and pork [3,4]. Vegetables and fruits also have been reported to be carriers in *Salmonella* transmission, and contamination can occur at multiple steps along the food chain [5]. Although there is a lack of official surveillance data for *Salmonella* in China, it is estimated that 22.2% of foodborne diseases are caused by *Salmonella* [6].

The increasing prevalence of multidrug resistance among *Salmonella* and resistance to clinically important antimicrobial agents has also been an emerging problem in China and other countries [7,8]. The spread of antimicrobial resistance potential in *Salmonella* is mainly attributed to integrons, which are genetic elements capable of capturing and transferring resistance genes among bacteria [9]. The class 1 integron is the most common type of integron identified in multidrug-resistant (MDR) *Salmonella*, and it plays an important role in the dissemination of resistance genes among pathogens [10].

To the best of our knowledge, there are several studies about antimicrobial resistance of foodborne *Salmonella* isolates in China [7,11,12]; however, similar research in Henan province is limited. Therefore, the objective of this study was to investigate the prevalence, serovars, antimicrobial resistance, and class 1 integrons of *Salmonella* in retail food in Henan province, China. We also examined the integron-positive isolates for the ability to transfer antimicrobial resistance genes via conjugation.

Methodology

Isolation and identification of Salmonella

Between July 2007 and August 2008, a total of 638 food samples including pork (n = 92), beef (n = 89), chicken (whole and parts, n = 95), mutton (n =

91), seafood (fish and shrimp, n = 73), vegetables (n = 98), and cooked meat products (sauced meats, roasted meats, and sausages, n = 100) were collected monthly from supermarkets and open-air markets in six cities in Henan province. The sample size was determined using the Sample Size Calculator (http://www.surveysystem.com/sscalc.htm) in order to ensure the reliability of the results. Henan, located in the central part of China, is a major agricultural center with about 104.9 million inhabitants. Due to its popularity in Henan as well as other provinces in China, meat – including raw meat and cooked meat products – accounted for the largest proportion of samples collected in this study. Isolation of *Salmonella* was performed using standard procedures described in the National Standard of the People's Republic of China (GB/T 4789.4-2003). Briefly, a rinse was performed by adding 25 g of food sample to 225 mL of buffer peptone water (BPW; Huankai Ltd., Guangzhou, Guangdong, China) in sterile lateral filter bags with thorough mixing by a homogenizer (BagMixer lab blender 400; Interscience, Saint-Nom-La-Breteche, France) and incubated at 37°C for 18 hours as pre-enrichment for *Salmonella*. Then 1 mL of the pre-enriched sample was inoculated into 10 mL of selenite cystine broth (SC; Huankai) and tatrathionate broth base (TTB; Huankai). Samples were incubated for 24 hours at 37°C (SC) and at 42°C (TTB), respectively. A loop of inoculum was streaked onto bismuth sulfite agar (BS; Huankai) and hektoen enteric agar (HE; Huankai) and incubated for 24 hours at 37°C. The presumptive isolates were picked from each plate and identified using the API 20E bacterial identification system (BioMerieux, Marcy l'Etoile, France).

Serotyping

Salmonella isolates were serotyped by the slide agglutination method using O and H antisera (Difco, Detroit, USA), according to the manufacturer's instructions.

Antimicrobial susceptibility testing

Each of the *Salmonella* isolates was tested for susceptibility to antimicrobials on Muller-Hinton agar (MH; Huankai) using the Kirby-Bauer disk diffusion method according to the recommendation of the Clinical and Laboratory Standards Institute (CLSI) [13]. Antimicrobial disks (Oxoid Ltd., Basingstoke, UK) with the following drug contents were used: ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), aztreonam (30 μg), piperacillin (100 μg), piperacillin/tazobactam (100/10 μg), ticarcillin/clavulanic acid (75/10 μg), ceftriaxone (30 μg), cefazolin (30 μg), cephalothin (30 μg), cefoperazone (75 μg), ceftazidime (30 μg), tetracycline (30 μg), chloramphenicol (30 μg), amikacin (30 μg), gentamycin (10 μg), streptomycin (10 μg), tobramycin (10 μg), sulfamethoxazole (300 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), norfloxacin (10 μg), and ciprofloxacin (5 μg). *Escherichia coli* ATCC 25922 and ATCC 35218 were used as reference strains. *Salmonella* isolates resistant to three or more classes of antimicrobials were defined as MDR isolates.

Detection of class 1 integrons and gene cassettes

Chromosomal DNA was obtained using the quick bacteria genomic DNA extraction kit (Dongsheng Biotech Co. Ltd., Guangzhou, Guangdong, China). The presence of the class 1 integron was detected by PCR targeting the class 1 integrase gene *intI1* using primers 5'-ACGAGCGCAAGGTTTCGGT-3' and 5'-GAAAGGTCTGGTCATACATG-3' as previously described [14]. Gene cassettes within the variable region of class 1 integron were then amplified using primers 5'-GGCATACAAGCAGCAAGC-3' and 5'-AAGCAGACTTGACCTGAT-3' as the described method [15]. The PCR products were sequenced at Invitrogen Biotechnology Co., Ltd. (Beijing, China). DNA sequence data were analyzed and aligned using BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST/).

Conjugation experiments

Conjugation was performed by the filter mating method as described previously [16]. Briefly, donor and recipient cells (1:1) were mixed in Luria-Bertani broth (LB; Huankai). The mixture was then collected on a sterilized 0.45 μm-pore-size filter and incubated on a blood agar (BA) plate at 37°C overnight. The mating mixture was washed from the filter and spread onto BA plates containing rifampicin (512 μg/mL) and streptomycin (50 μg/mL). Transconjugants were confirmed to be *E. coli* by the API test and the class 1 integron was detected by PCR using primers shown above.

Results

A total of 62 *Salmonella* isolates were isolated from various food samples examined in this study. The overall isolation rate of *Salmonella* in the retail foods was 9.7%. The distribution of the isolates from a variety of food products is presented in Table 1. The *Salmonella* isolates were found most frequently in

Table 1. Prevalence of *Salmonella* in food samples collected in Henan province of China

Food type	No. of samples	No. of positive samples (%)
Raw meat	367	46 (12.5)
Pork	92	14 (15.2)
Beef	89	15 (16.8)
Chicken	95	7 (7.4)
Mutton	91	10 (11.0)
Seafood	73	5 (6.8)
Vegetables	98	2 (2.0)
Cooked meat products	100	9 (9.0)
Total	638	62 (9.7)

beef (16.8%), followed by pork (15.2%). Sixteen different serovars were identified among the 62 isolates, and one isolate from pork sample was untypable (Table 2). The top five serovars were *S.* Anatum (n = 14, 22.6%), *S.* Senftenberg (n = 11, 17.7%), *S.* Derby (n = 8, 12.9%), *S.* Enteritidis (n = 5, 8.1%), and *S.* Choleraesuis (n = 4, 6.4%).

The results of resistance analysis of the *Salmonella* strains against 21 antimicrobial agents are presented in Table 3. Out of 62 *Salmonella* isolates tested, 58 (93.5%) were resistant to one or more antimicrobials, while only 4 (6.5%) were fully susceptible. No resistance was observed to amoxicillin/clavulanic acid, cephalothin, and gentamycin. Resistance to sulfamethoxazole (90.3%) was observed most frequently, followed by resistance to trimethoprim/sulfamethoxazole (87.1%), streptomycin (29.0%), and ciprofloxacin (25.8%), whereas resistance to β-lactams was observed less frequently. Thirty-three (53.2%) *Salmonella* isolates were resistant to 1-3 antimicrobials, 17 (27.4%) to 4-6 antimicrobials, and 8 (12.9%) to 7-9 antimicrobials. Among the 21 (33.9%) MDR isolates of *Salmonella*, *S.* Derby (n = 5) and *S.* Enteritidis (n = 4) serovars were observed most often (Table 4).

Class 1 integrons were detected in 10 *Salmonella* isolates, only one of which was negative for the resistance gene cassette (Table 4). The remaining nine *intI1*-positive isolates contained three groups of resistance gene cassette, consisting of *dfrA12-aadA2* (2.0 k, n = 5), *dfrA1-aadA1* (1.5 k, n = 3), and *dfrA1* (1.2 k, n = 1). Three *Salmonella* isolates could transfer their integrons and resistance genes to *E. coli* by conjugation. The results indicated the transconjugants obtained had a plasmid carrying the class 1 integron and the transfer frequency was in the range of 2.4×10^{-6} to 8.0×10^{-5} transconjugant per recipient cell.

Discussion

In the current study, we examined *Salmonella* isolates recovered from retail food purchased in Henan province. The prevalence of *Salmonella* in food products of other provinces in China was 3.5% in Jiangsu, 20.9% in Hebei, and 36.8% in Shaanxi [7,11,17]. Our results not only provide information about the prevalence of foodborne *Salmonella* in Henan, but also provide a better understanding of the differences in contamination by *Salmonella* among the different provinces in China. Previous studies showed that the prevalence of *Salmonella* in chicken was highest [11], while in our study, the isolates from beef samples constituted the largest proportion. However, it was difficult to compare the data on the prevalence of *Salmonella* in different studies, because the prevalence may be affected by diversity in sampling methods, sampling seasons, and isolation procedures. Moreover, we also found that the level of *Salmonella* contamination in cooked meat products was much higher (9.0%). Given that these products are ready-to-eat foods that are consumed without further cooking or processing, they may be an increasing cause of enteric diseases [18].

Among these isolates, *S.* Anatum and *S.* Senftenberg were most common; however, *S.* Senftenberg was not found in the previous report about the occurrence of *Salmonella* serovars isolated from food in Henan province [19]. In the same area, the distribution of serovars may be varied due to the different food types collected. *S.* Derby and *S.* Enteritidis were commonly identified serovars in the present study. Furthermore, the two serovars were also frequently observed in clinical *Salmonella* in Henan [20], suggesting an association between *Salmonella*-contaminated food and salmonellosis.

Table 2. Distribution of *Salmonella* serovars in retail food

Serotype	No. of isolates							
	Pork	Beef	Chicken	Mutton	Seafood	Vegetables	Cooked meats	Total (%)
Agona	2	1						3 (4.8)
Anatum	1	4	1	4	3		1	14 (22.6)
Berta							1	1 (1.6)
Choleraesuis	2	1			1			4 (6.4)
Derby	1	2	1	2		1	1	8 (12.9)
Enteritidis		1	2	1			1	5 (8.1)
Irumu				1				1 (1.6)
Kentucky			1					1 (1.6)
Lomita		2						2 (3.2)
London	1			1	1			3 (4.8)
Oranienburg	1							1 (1.6)
Saintpaul			2					2 (3.2)
Senftenberg	4	3				1	3	11 (17.7)
Sinstorf	1	1						2 (3.2)
Stanley							1	1 (1.6)
Typhimurium				1			1	2 (3.2)
Untypable	1							1 (1.6)
Total	14	15	7	10	5	2	9	62 (100)

Table 3. Antimicrobial resistance of *Salmonella* isolated from retail food

Antimicrobial	No. of isolates (%)		
	R	I	S
β-lactams			
Ampicillin	8 (12.9)	0	54 (87.1)
Amoxicillin/clavulanic acid	0	0	62 (100)
Aztreonam	4 (6.5)	0	58 (93.5)
Piperacillin	6 (9.7)	0	56 (90.3)
Piperacillin/tazobactam	0	3 (4.8)	59 (95.2)
Ticarcillin/clavulanic acid	3 (4.8)	0	59 (95.2)
Ceftriaxone	1 (1.6)	3 (4.8)	58 (93.5)
Cefazolin	0	1 (1.6)	61 (98.4)
Cephalothin	0	0	62 (100)
Cefoperazone	1 (1.6)	4 (6.5)	57 (91.9)
Ceftazidime	3 (4.8)	0	59 (95.2)
Tetracycline	11 (17.7)	0	51 (82.3)
Chloramphenicol	13 (21.0)	0	49 (79.0)
Aminoglycosides			
Amikacin	2 (3.2)	0	60 (96.8)
Gentamycin	0	0	62 (100)
Streptomycin	18 (29.0)	0	44 (71.0)
Tobramycin	0	1 (1.6)	61 (98.4)
Sulfonamides			
Sulfamethoxazole	56 (90.3)	0	6 (9.7)
Trimethoprim/sulfamethoxazole	54 (87.1)	0	8 (12.9)
Quinolones			
Norfloxacin	4 (6.5)	0	58 (93.5)
Ciprofloxacin	16 (25.8)	0	46 (74.2)

Table 4. Antimicrobial resistance characteristics of MDR *Salmonella* isolates recovered from retail food

Strain	Source	Serotype	Resistance or intermediate susceptibility[a]	*intI1*	Size of integron (kb)	Gene cassette	Conjugation rate
07S103	Beef	Anatum	AMP, STR, SUL, SXT	+	2.0	*dfrA12-aadA2*	
07S106	Pork	Choleraesuis	CIP, SUL, SXT, TET	+	1.2	*dfrA1*	
07S109	Mutton	Derby	CHL, CIP, SUL, SXT				
08S021	Cooked meats	Derby	CHL, STR, SUL, SXT				
07S098	Beef	Derby	STR, SUL, SXT, TET	+	1.5	*dfrA1-aadA1*	
07S087	Pork	Derby	CFP, CHL, STR, SUL, SXT, TET, TZP	+	1.5	*dfrA1-aadA1*	
07S095	Beef	Derby	CHL, CRO, PIP, STR, SUL, SXT, TET, TZP	+			
08S016	Mutton	Enteritidis	AMP, CHL, STR, SUL, TET				
08S013	Beef	Enteritidis	AMP, CHL, STR, SUL, SXT, TET, TZP	+	2.0	*dfrA12-aadA2*	
08S115	Chicken	Enteritidis	AMP, CHL, STR, SUL, SXT, TET	+	1.5	*dfrA1-aadA1*	5.6×10^{-6}
08S114	Chicken	Enteritidis	AMP, CHL, CIP, STR, SUL, SXT, TET	+	2.0	*dfrA12-aadA2*	8.0×10^{-5}
07S139	Chicken	Kentucky	AZT, CAZ, CFP, CIP, CRO, PIP, STR, SUL, SXT				
07S102	Beef	Lomita	AZT, CIP, CRO, SUL, SXT				
07S096	Mutton	London	AMK, AZT, CAZ, CIP, SUL, SXT, TOB				
08S019	Pork	London	AMK, AZT, CAZ, CHL, SUL, TCC				
08S098	Chicken	Saintpaul	AMP, SUL, SXT, TET				
07S130	Chicken	Saintpaul	STR, SUL, SXT, TET				
07S104	Beef	Senftenberg	CIP, STR, SUL, SXT				
07S075	Cooked meats	Senftenberg	CIP, STR, SUL, SXT				
07S127	Mutton	Typhimurium	AMP, CFP, CHL, PIP, STR, SUL, SXT,	+	2.0	*dfrA12-aadA2*	2.4×10^{-6}
08S027	Cooked meats	Typhimurium	AMP, CFP, CHL, CIP, PIP, STR, SUL, SXT, TET	+	2.0	*dfrA12-aadA2*	

[a]AMP, ampicillin; STR, streptomycin; SUL, sulfamethoxazole; SXT, trimethoprim/sulfamethoxazole; CIP, ciprofloxacin; TET, tetracycline; CHL, chloramphenicol; CFP, cefoperazone; TZP, piperacillin/tazobactam; CRO, ceftriaxone; PIP, piperacillin; AMK, amikacin; AZT, aztreonam; CAZ, ceftazidime; TOB, tobramycin; TCC, ticarcillin/clavulanic acid

In China, the predominant serovar of foodborne *Salmonella* was *S.* Derby, while *S.* Typhimurium was the main serovar isolated from humans [21]. The difference in dominant serovars between foodborne and clinical isolates may be due to differences in pathogenicity and resistance profiles of the two serovars [22].

Compared to the previous studies conducted in Henan as well as other provinces in China, *Salmonella* isolates recovered from retail food in our study showed a higher resistance to trimethoprim/sulfamethoxazole and ciprofloxacin [11,19]. Almost all the isolates were resistant to at least one antimicrobial, and nearly 35.0% were MDR isolates. The high prevalence of antimicrobial resistance from retail food was also reported by other studies [12,23]. The use of antimicrobials in food animals for disease treatment and growth promotion may potentially lead to the emergence of antimicrobial-resistant pathogens [24]. The increasing prevalence of resistant *Salmonella* in China and other countries presents an enormous challenge to the treatment of *Salmonella* infections in humans and animals [9,11,20,25].

All *intI1*-positive isolates were MDR strains, which supported the hypothesis of an association between the presence of class 1 integrons and emerging MDR in *Salmonella* [10]. Our results showed that three *Salmonella* isolates could transfer their integrons and resistance genes to *E. coli* by conjugation. Therefore, it is concluded that the class 1 integron was located on a conjugative plasmid in these isolates. Previous reports have also suggested that most of the resistance determinants and class 1 integrons in *Salmonella* isolates were encoded in a transferable plasmid, which might be transferred to the same or different bacterial species by conjugation [23].

Conclusion

Our study illustrates a potential public health risk of *Salmonella* in Henan because of its presence in various food items, particularly in raw meats and cooked meat products. Efforts that include further implementation of hazard analysis of critical control point programs in food production are needed to reduce the incidence of *Salmonella* in food. The high rate of MDR *Salmonella* isolates and the presence of integrons in this study suggest that effective measures should be taken to facilitate the reasonable use of antimicrobials in both human and veterinary medicine. The monitoring of antimicrobial resistance among foodborne *Salmonella* is important because the resistance determinants could be spread from food products to humans by transferable elements.

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