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Short Communication

Prevalence and antimicrobial resistance of *Salmonella* in retail foods in northern China

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ABSTRACT

A total of 387 retail meat, seafood and milk powder samples were collected from nine cities in northern China in 2005 and screened for the presence of *Salmonella*. *Salmonella* strains isolated were subjected to serotyping and antimicrobial susceptibility testing. *Salmonella* was isolated from 81 (20.9%, 81/387) samples and classified into 23 serotypes. The isolates were frequently resistant to sulfamethoxazole (86.4%), sulfamethoxazole/trimethoprim (48.1%), nalidixic acid (30.9%), tetracycline (19.8%), carboxybenzylpenicillin (17.3%), amoxicillin (17.3%) and ampicillin (16.0%). The multiple resistance (resistance to ≥3 antibiotics) was found in 29.6% (n = 24) isolates. Additionally, 4 isolates from chicken displayed the ACSSuTNx profile, resistant to ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline and nalidixic acid, in particular, strain HBS084 showing the resistance to as many as 20 antibiotics. *Salmonella* from chicken showed the higher frequency of antimicrobial resistance. Our findings indicate that in northern China food products of animal origin can be a source of exposure for consumers to multiresistant *Salmonella* strains.

1. Introduction

Salmonella that includes more than 2500 different serotypes represents a leading cause of foodborne infections worldwide (Chen et al., 2004; Magistrali et al., 2008; White et al., 2002). The majority of the infections are associated with ingestion of contaminated foods such as poultry, beef, pork, egg, milk, cheese, seafood, fruits, juice and vegetables (Brands et al., 2005; Zhao et al., 2008).

Salmonella gastroenteritis is generally self-limiting illness, but severe cases in immuno-compromised individuals, elderly persons or neonates, and systemic infections may require effective chemotherapy (Lee et al., 1994). Currently the increasing prevalence of multidrug resistance among *Salmonella* and resistance to clinically important antimicrobial agents such as fluoroquinolones and third-generation cephalosporins has also been an emerging problem in China and other countries (Brands et al., 2005; Chao et al., 2007; Gebreyes and Thakur, 2005). Additionally, multidrug-resistant *Salmonella* have been isolated, and they are in many serotypes, such as Agona, Anatum, Choleraesuis, Derby, Dublin, Heidelberg, Kentucky, Newport, Pullorum, Schwarzengrund, Senftenberg, Typhimurium and Uganda (Chen et al., 2004; Gebreyes et al., 2004; Gebreyes and Thakur, 2005; Pan et al., 2009; Zhao et al., 2008). Therefore, the particular concern is severity of the multidrug resistance in *Salmonella*. The levels of resistance are varied and influenced by antimicrobial use to humans and animals, as well as the geographical differences. In China, a major producer and consumer of animal source foods, the per capita consumption of meats and seafood has increased significantly over the past century. With this increase in consumption of food products of animal origin, it also comes with the increased potential for exposure to foodborne pathogens through the food chain. According to the foodborne diseases outbreaks report which was released by the National Foodborne Diseases Surveillance Network in China, during 1992–2005, among bacterial foodborne illness outbreaks, salmonellosis is the second leading cause, and approximately 10–20% of the outbreaks were caused by *Salmonella* annually (Chen et al., 2008; Liu et al., 2004, 2006, 2008).

Historically, most studies on the prevalence and characterization of the antimicrobial resistance in *Salmonella* have been restricted to the isolates from clinical and/or veterinary sources (Khan et al., 2009; Randall et al., 2004). Information on the potential role of food samples in dissemination of multidrug-resistant *Salmonella* in China is very limited. In the present study, we reported prevalence, serotypes, and antibiotic resistance patterns of *Salmonella* strains isolated from food products of animal origin in nine cities of Hebei province in the northern China in 2005. Our overall aim was to clarify the correlation of the antimicrobial resistance profiles, serotypes and isolation

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sources, and to identify the most likely food sources responsible for human salmonellosis outbreak in China.

2. Materials and methods

2.1. Collection of food samples and isolation of Salmonella

Salmonella isolates were obtained through the National Active Foodborne Pathogens Surveillance System. As shown in Table 1, a total of 387 food samples including pork (n=45), chicken (n=120), beef (n=45), mutton (n=45), seafood (n=96) and milk powder (n=36) were randomly collected in open-air markets and large supermarkets of nine surveillance points in Hebei province, located in the northern part of China in 2005. The strains were independently isolated from different and individual food samples.

Salmonella isolation and identification were performed as described previously (Chen et al., 2004; Dziadkowiec et al., 1995), with some modifications. Briefly, each sample (25 g) was placed in separate sterile plastic bags and washed with 225 ml buffered peptone water (BPW) with shaking vigorously for 2 min. The rinse was incubated at 37 °C in a water bath with shaking at 100 rpm for 8 h, then 10 ml of buffered peptone water was added to 100 ml selenite cystine broth at 37 °C for 24 h. A loop of inoculum from the selenite cystine broth was streaked onto bismuth sulfite agar and Hektoen enteric agar and incubated for 24 h at 37 °C. A minimum of two of presumptive *Salmonella* colonies were picked from each plate and stabbed into triple sugar iron and lysine–iron agar slants, respectively, incubated for 24 h at 36 °C. Isolates with positive slant reactions were then tested for agglutination with ATB ID 32E, which is a standardized system for the identification of *Enterobacteriaceae* and other nonfastidious gram negative rods, and the result was determined automatically using the ATB Expression system (BioMérieux, Lyon, France).

Salmonella isolates were stored in Luria–Bertani (LB) broth containing 15% glycerol at − 80 °C until use.

2.2. Serotyping

According to the manufacturer's instructions, serotyping of *Salmonella* isolates was carried out with the antisera available commercially (Difco, Detroit, MI, USA).

2.3. Susceptibility testing

A total of 28 antimicrobial agents currently used in the veterinary and medical therapy were used. By the Kirby–Bauer disk diffusion method, the isolates were tested against ampicillin (10 μg), piperacillin (100 μg), carboxybenzylpenicillin (100 μg), amoxicillin (20 μg), cefazolin (30 μg), cephalothin (30 μg), ceftriaxone (30 μg), cefoperazone (75 μg), ceftazidime (30 μg), amoxicillin/clavulanic acid (20/10 μg), piperacillin/tazobactam (100/10 μg), ticarcillin/clavulanic acid (75/10 μg), ampicillin/sulbactam (10/10 μg), cefoperazone/sulbactam (75/75 μg), aztreonam (30 μg), gentamicin (10 μg), amikacin (30 μg), streptomycin (10 μg), tobramycin (10 μg), nitrofurantoin (300 μg), sulfamethoxazole (300 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), nalidixic acid (30 μg), norfloxacin (10 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), tetracycline (30 μg), and chloramphenicol (30 μg). The classes of the resistance level were defined as described by the Clinical and Laboratory Standards Institute (CLSI, 2005) and indicated as susceptible (S), intermediate (I) or resistance (R). *Escherichia coli* strain ATCC 25922 and ATCC 35218 were used as control strains.

Table 1
Occurrence of *Salmonella* in selected food samples in Hebei province of China, 2005.

Food type	No. of positive samples/No. of total sample (%)
Retail meat	61/255 (23.9)
Pork	12/45 (26.7)
Chicken	19/120 (15.8)
Beef	15/45 (33.3)
Mutton	15/45 (33.3)
Seafood	20/96 (20.8)
Milk powder	0/36 (0)
Total	81/387 (20.9)

Table 2
Salmonella serotypes isolated from the food products in Hebei province, China in 2005.

Serotype	No.	%
Agona	11	13.6
Senftenberg	8	9.9
Meleagridis	7	8.6
Derby	7	8.6
Irumu	6	7.4
Choleraesuis	5	6.2
London	4	4.9
Enteritidis	4	4.9
Manhattan	4	4.9
Saintpaul	3	3.7
Indiana	3	3.7
Sinstorf	3	3.7
Newlands	2	2.5
Abony	2	2.5
Muenster	2	2.5
Calabar	2	2.5
Poona	2	2.5
Anatum	1	1.2
Bredene	1	1.2
Kingston	1	1.2
Thompson	1	1.2
Montevideo	1	1.2
Untypable	1	1.2
Total	81	100

2.4. Statistical analysis

The statistical package SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used, and the P value less than 0.05 was considered significant.

3. Results and discussion

3.1. Prevalence of Salmonella in retail food products

A total of 81 *Salmonella* isolates were recovered, representing 20.9% of samples tested (Table 1). The prevalence of *Salmonella* in food products of other provinces in China was 2.32% in 2000 (Wang et al., 2002), 3.32% in 2001, and 3.55% in 2002 (Wang et al., 2004). The relatively higher isolation rate (23.9%) from meats observed in the present study is consistent with previous reports from China as well as other parts of the world (Chen et al., 2004; Chao et al., 2007; Van et al., 2007; Zhao et al., 2008). These data can also support the previous reports indicating that retail meat accounted for the largest percentage (10–60%) of all food poisoning related outbreaks of *Salmonella* infections in China (Chen et al., 2008; Liu et al., 2004, 2006, 2008). Moreover, we have also found the levels of *Salmonella* contamination in seafood samples were much higher (20.8%), indicating another potential cause of higher rate of enteric diseases. No *Salmonella* was isolated from milk powder. Previous reports have also suggested that milk and its products accounted for less than 5% of all food poisoning related outbreaks of *Salmonella* infections in China (Chen et al., 2008; Liu et al., 2004, 2006, 2008).

3.2. Serotyping and antibiotics susceptibility

The results of the serotyping and antibiotic resistance tests of 81 *Salmonella* isolates are shown in Tables 2 and 3, respectively. The

Table 3
Percentages of *Salmonella* isolates resistant to each antimicrobial from various food sources.

Antibiotic	Number of resistant and intermediate susceptibility isolates (%) from:											
	Pork (*n* = 12)		Chicken (n = 19)		Beef (n = 15)		Mutton (n = 15)		Seafood (n = 20)		All sources (n = 81)	
β-lactams												
Ampicillin	2 (16.7)	0	9 (47.4)	0	0	0	0	0	2 (10.0)	0	13 (16.0)	0
Piperacillin	1 (8.3)	3 (25.0)	7 (36.8)	4 (21.1)	0	1 (6.7)	0	1 (6.7)	3 (15.0)	6 (30.0)	11 (13.6)	15 (18.5)
Carboxybenzylpenicillin	2 (16.7)	7 (58.3)	9 (47.4)	8 (42.1)	0	9 (60.0)	1 (6.7)	7 (46.7)	2 (10.0)	14 (70.0)	14 (17.3)	45 (55.6)
Amoxicillin	2 (16.7)	0	9 (47.4)	0	1 (6.7)	0	0	0.0	2 (10.0)	0	14 (17.3)	0
Cefazolin	0	0	1 (5.3)	1 (5.3)	1 (6.7)	0	0	0	0	0	2 (2.5)	1 (1.2)
Cephalothin	0	1 (8.3)	1 (5.3)	0	0	0	0	0	0	1 (5.0)	1 (1.2)	2 (2.5)
Ceftriaxone	0	0	0	2 (10.5)	0	0	0		0	0	0	2 (2.5)
Cefoperazone	0	0	0	2 (10.5)	0	0	0	0	0	2 (10.0)	0	4 (4.9)
Ceftazadime	1 (8.3)	0	1 (5.3)	0	0	0	0	0	0	0	2 (2.5)	0
Amoxicillin-clavulanate	0	0	2 (10.5)	2 (10.5)	0	0	0	0	0	0	2 (2.5)	2 (2.5)
Piperacillin/tazobactam	0	1 (8.3)	0	0	0	1 (6.7)	0	3 (20.0)	0	7 (35.0)	0	12 (14.8)
Ticarcillin/clavulanic acid	0	0	0	1 (5.3)	0	0	0	0	1 (5.0)	0	1 (1.2)	1 (1.2)
Ampicillin/Sulbactam	0	0	3 (15.8)	6 (31.6)	0	0	0	0	1 (5.0)	0	4 (4.9)	6 (7.4)
Cefoperazone/Sulbactam	0	0	0	1 (5.3)	0	0	0	0	0	2 (10.0)	0	3 (3.7)
Aztreonam	0	0	0	0	0	0	0	0	0	0	0	0
Aminoglycosides												
Gentamicin	0	0	6 (31.6)	0	0	0	0	0	1 (5.0)	0	7 (8.6)	0
Amikacin	0	0	3 (15.8)	0	0	0	0	0	0	0	3 (3.7)	0
Streptomycin	0	6 (50.0)	7 (36.8)	7 (36.8)	0	7 (46.7)	1 (6.7)	7 (46.7)	1 (5.0)	2 (10.0)	9 (11.1)	29 (35.8)
Tobramycin	0	1 (8.3)	7 (36.8)	0	0	0	0	0	1 (5.0)	1 (5.0)	8 (9.9)	2 (2.5)
Nitrofurans												
Nitrofurantoin	0	4 (33.3)	4 (21.1)	4 (21.1)	0	1 (6.7)	0	3 (20.0)	1 (5.0)	2 (10.0)	5 (6.2)	14 (17.3)
Sulfonamides												
Sulfamethoxazole	10 (83.3)	0	17 (89.5)	2 (10.5)	13 (86.7)	1 (6.7)	11 (73.3)	1 (6.7)	19 (95.0)	0	70 (86.4)	4 (4.9)
Trimethoprim-sulfamethoxazole	6 (50.0)	0	11 (57.9)	0	5 (33.3)	0	4 (26.7)	1 (6.7)	13 (65.0)	0	39 (48.1)	1 (1.2)
Quinolones and fluoroquinolone												
Nalidixic acid	6 (50.0)	1 (8.3)	14 (73.7)	1 (5.3)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	3 (15.0)	3 (15.0)	25 (30.9)	7 (8.6)
Norfloxacin	0	0	8 (42.1)	0	0	0	0	0	0	0	8 (9.9)	0
Ciprofloxacin	0	0	8 (42.1)	0	0	0	0	0	0	1 (5.0)	8 (9.9)	1 (1.2)
Ofloxacin	0	0	5 (26.3)	2 (10.5)	0	0	0	0	0	0	5 (6.2)	2 (2.5)
Tetracycline	4 (33.3)	0	9 (47.4)	1 (5.3)	0	1 (6.7)	1 (6.7)	0	2 (10.0)	0	16 (19.8)	2 (2.5)
Chloramphenicol	2 (16.7)	1 (8.3)	8 (42.1)	0	0	0	0	0	0	2 (10.0)	10 (12.3)	3 (3.7)

resistance to multiple antimicrobial agents was predominantly seen in Derby, Indiana and Saintpaul serotypes (Table 5). Additionally, resistant phenotypes appear to be associated with particular serotypes, for example, all isolates of *S.* Indiana and *S.* Saintpaul isolates were multiresistant to five classes of antibiotics, including β-lactams, sulfonamides, fluoroquinolones, chloramphenicol and tetracycline. Due to the limited number of isolates from foods, it is difficult to assess evidently the relationship between serotype and multiresistance.

Resistance to sulfamethoxazole, sulfamethoxazole/trimethoprim and nalidixic acid was very common, this finding is in agreement with studies from China and other countries (Chen et al., 2004; White et al., 2001, 2002; Pan et al., 2009; Van et al., 2007; Zhao et al., 2008). Nalidixic acid resistance was especially prevalent in isolates from chicken meat. Previous studies showed that *Salmonella* was predominant in chickens and particularly resistant to quinolones including nalidixic acid since 2000 (Cheong et al., 2007; Padungtod and Kaneene, 2006). These findings may not be surprising as trimethoprim in combination with sulfamethoxazole has been used for 30 years in human and veterinary medicine (Poros-Gluchowska and Markiewicz, 2003), and quinolones and fluoroquinolones are being used broadly in veterinary medicine in China since 1980s (Chen et al., 2004).

In the present study, less than 5% of strains were resistant to first generation (cefazolin and cephalothin) and third-generation cephalosporins (ceftazadime). Other reports describe a decreasing susceptibility of *Salmonella* strains from food products and veterinary sources to these antimicrobials (Chao et al., 2007; Pan et al., 2009).

In the current study, *Salmonella* isolated from chicken showed a greater degree of multiresistance than that from seafood and other meats ($p = 0.0002$) (Tables 4 and 5). It was observed that 9 isolates, 8 from chicken and 1 from seafood, were resistant to at least 10 antimicrobials, including 4 isolates (HBS121, HBS145, HBS138, and HBS084) from chicken revealing the ACSSuTNx profile (resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline, and nalidixic acid). Interestingly, strain HBS084, of which serotype is Indiana, showed the resistance to as many as 20 antibiotics (Table 5).

In conclusion, food products of animal origin may pose a risk in serving as reservoirs and disseminating resistant *Salmonella* in Hebei province of China**.** To reduce the risk of *Salmonella* infection, this will require close cooperation between sectors involved in food hygiene, prevention and control of diseases transmitted from animals to humans, hospital infection control, resistance monitoring and prudent use of antimicrobials in humans and animals. Additionally, consumers should be very careful for high-risk foods, and take proper care for prevention of the growth of the microorganisms, e.g., short-term

Table 4
Occurrence of antimicrobial drug resistance in *Salmonella* isolates by source of isolation, Hebei province, China in 2005.

Number of antibiotic	Number (%) of resistant to from:					
	Pork (*n* = 12)	Chicken (n = 19)	Beef (n = 15)	Mutton (n = 15)	Seafood (n = 20)	All sources (n = 81)
0	2 (16.7)	0	0	3 (20.0)	0	5 (6.2)
1	1 (8.3)	2 (10.5)	8 (53.3)	8 (53.3)	5 (25.0)	24 (29.6)
2	4 (33.3)	6 (31.6)	6 (40.0)	2 (13.3)	9 (45.0)	27 (33.3)
3	1 (8.3)	1 (5.3)	1 (6.7)	1 (6.7)	2 (10.0)	6 (7.4)
4	2 (16.7)	1 (5.3)	0	1 (6.7)	1 (5.0)	5 (6.2)
5-9	2 (16.7)	1 (5.3)	0	0	1 (5.0)	4 (4.9)
10 or + drugs	0	8 (42.1)	0	0	1 (5.0)	9 (11.1)

Table 5
Antibiotic resistance and serotype of all multiresistant *Salmonella* isolates.

Isolate	Source[a]	Serotype	Resistance or intermediate susceptibility[b] to:																											
			AMP	PIP	AM	AMX	CZ	CEP	CRO	CFP	CAZ	AMX/CA	PIP/TA	TIC/CA	AMC/SU	CFP/SU	AZT	GEN	AMK	STR	TOB	FT	SMX	SXT	NAL	NOR	CIP	OFL	TET	CHL
HBS002	a	Newlands(e1)		I	I																		R	R	R				R	
HBS017	a	Derby(B)			I								I							R			R		R					R
HBS029	a	London(e1)			I						R									I	I	I	R	R	I					
HBS012	a	Derby(B)	R	I	R	R																I	R	R	R				R	I
HBS010	a	Derby(B)	R	R	R	R														I		I	I	R	R					R
HBS057	b	Irumu(c1)					R																R	R						
HBS099	c	Meleagridis(e1)			I		I			I										I		R	R	R	R				R	
HBS163	c	Senftenberg(e1)	R	R	R	R									I							R	R		R				R	
HBS121	c	Saintpaul(B)	R	I	R	R						R			I			R		R	R		R	R	R	R	R	R	R	R
HBS136	c	Saintpaul(B)	R	I	R	R						I			R						R		R	R	R	R	R	R	R	R
HBS143	c	Saintpaul(B)	R	R	R	R									I			R	R	I	R	I	R	R		R	R		R	R
HBS117	c	Agona(B)	R	R	R	R									I							R	R	R	R	R	R	R	R	R
HBS145	c	Agona(B)	R	R	R	R									I			R	R	R	R		R	R	R	R	R	I	R	R
HBS138	c	Indiana(B)	R	R	R	R						I		I	R			R		R	R		R	R	R	R	R	R	R	R
HBS146	c	Indiana(B)	R	R	R	R									I			R	R	I	R	I	R	R	R	R	R	I	R	R
HBS084	c	Indiana(B)	R	R	R	R	R	R	I	I	R	R			R	I		R		R	R		R	R	R	R	R	R	R	R
HBS511	c	Untypable																		I			R		R				R	
HBS219	d	Meleagridis(e1)		I	R								I										R	R						
HBS196	d	Enteritidis(D)											I							R			R	I	R				R	
HBS339	e	Derby (B)			I								I							R			R	R	R				R	
HBS363	e	Agona (B)		R	I			I		I			I			I							R	R						
HBS357	e	Senftenberg(e1)		I																		I	R	R	R					I
HBS380	e	Montevideo(c1)	R	R	R	R										I		R		R	I	I	R		R				R	
HBS371	e	Choleraesuis(c1)	R	R	R	R							I	R	R					R	R	R	R	R	I		I		R	I

[a] Source abbreviations are as follows: a, pork; b, beef; c, chicken; d, mutton; e, seafood.
[b] AMP, ampicillin; PIP, piperacillin; AM, carboxybenzylpenicillin; AMX, amoxicillin; CFZ, cefazolin; CEP, cephalothin, CRO, ceftriaxone; CFP, cefoperazone; CAZ, ceftazidime; AUG, amoxicillin/clavulanic acid; PIP/TA, piperacillin/tazobactam; TICC/CA, ticarcillin/Clavulanic acid, AMC/SU, ampicillin/sulbactam; CFP/SU, cepfoperazone/Sulbactam; AZT, aztreonam; GEN, gentamicin; AMI, amikacin; STR, streptomycin; TOB, tobramycin; FT, nitrofurantoin; SMX, sulfamethoxazole; SXT, trimethoprim-sulfamethoxazole; NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; OFL, ofloxacin; TET, tetracycline; CHL, chloramphenicol.

refrigerated storage of perishable foods and cooking before consumption. For better understanding of *Salmonella* contamination sources and prevention strategies, a large-scale future study is required in particular endemic areas with samples collected from the consumers, retail food shops, food-processing plants as well as from other natural sources. Therefore a holistic management approach may be needed to significantly reduce the overall burden of *Salmonella* on human health.

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