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Prevalence and Antimicrobial Resistance of *Salmonella* sp. Isolated from Domestic Animals in Eastern China

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Abstract: A total of 163 *Salmonella* sp. isolates representing 15 serotypes recovered from faecal samples of domestic animals (chicken, duck, goose and pig) in eastern China during 2008-2009 were tested for antimicrobial susceptibilities. *S.* Senftenberg, *S. Typhimurium S. Pullorum and S. Enteritidis* were the most prevalent serovars. Resistance was most often observed to carbenicillin (65.4%), followed by nalidixic acid (48.8%), tetracycline (46.9%), sulfafurazole (45.7%), ampicillin (43.2%), streptomycin (38.3%) and trimethoprim/sulfamethoxazole (33.3%). With regards to the source of isolates, chicken *Salmonella* sp. isolates displayed the highest rate of resistance being resistant to at least one antimicrobial (100%) followed by those recovered from pig (93.4%), goose (90.7%) and duck (80%). Serovars commonly showing Multidrug Resistance (MDR) to >9 antimicrobials were *S. Enteritidis* (55.6%), *S. Pullorum* (17.9%) and *S. Typhimurium* (17.2%). This study has revealed the prevalence and antimicrobial resistance patterns of *Salmonella* sp. in domestic animals in eastern China and provides the important information for better controlling these pathogens.

Key words: Antimicrobial resistance, domestic animals, eastern China, *Salmonella* sp., multidrug resistance, susceptibilities

INTRODUCTION

Salmonella is a genus of bacteria that are a major cause of foodborne illness throughout the world. The reservoirs of *Salmonella* are considered to be domestic animals, particularly poultry and pigs and these organisms are easily isolated from the feces (Vo *et al*., 2006). These carrier animals likely play an important role in the spread of infection between herds and flocks and consequently serve as sources of food contamination and human infection (Carrique-Mas *et al*., 2008).

In China, during 1994-2005, a total of 57612 outbreaks of foodborne disease were reported and *Salmonella* was the most identified agent, accounting for 22.2% of illnesses (Wang *et al*., 2007). Salmonellosis in the food animal industry can be treated and controlled with antimicrobial therapy. However, the control of *Salmonella* infections is difficult because increasing antimicrobial resistance has been reported in animal and human species (Gebreyes and Thakur, 2005; M'ikanatha *et al*., 2010). Zhao *et al*. (2007) reported that 82% of 380 animal isolates of *Salmonella* were resistant to at least one antimicrobial drug, with 52% exhibiting resistance to five or more antimicrobial drugs. In China, there are few reports regarding the prevalance of *Salmonella* sp. in live animals and the antimicrobial resistance of the isolates (Liu *et al*., 2010). Therefore the objectives of the present study were to investigate the prevalance of *Salmonella* sp. in chickens, ducks, geese and pigs in the farm in eastern China and to characterize the antimicrobial resistance of the isolates.

MATERIALS AND METHODS

Sampling: The animal isolates were collected from chickens, ducks, geese and pigs (Table 1) in 7 provinces of in eastern China during the year 2008-2009. Faecal samples from healthy or sick animals were taken on farms. The animals sampled came from different flocks or herds. About 10 g of faeces was collected from each animal and placed in a sterile sampling bag, kept in an ice-box at 4-8°C and transported to the laboratory within 24 h. About 10-20 samples were taken at each animal farm. Samples were analysed at Jiangsu Key Laboratory of Zoonosis.

Isolation and identification of ***Salmonella*****:** Faecal samples were inoculated into buffered peptone water

Table 1: Prevalence of *Salmonella* in domestic animals in eastern China

Animal species	No. of samples	No. positive for Salmonella (%)	No. investigated isolates	
			Healthy	Diseased
Chicken	681	33 (4.8)	9	24
Duck	285	15 (5.3)	15	0
Goose	505	54 (10.7)	54	0
Pig	615	61 (9.9)	53	8

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(Difco) for enrichment at a ratio of 1 g faeces to 10 mL of broth. After incubation at 42°C for 18 h, the broth was inoculated onto desoxycholate hydrogen sulphide lactose and brilliant green agar plates (Hangzhou Microbial Reagent Co., Ltd., Hangzhou, China), each supplemented with 20 mg L^{-1} of novobiocin sodium (Hangzhou Microbial Reagent Co., Ltd.) and incubated at 37°C for 18 h. Candidate colonies were identified biochemically by triple sugar iron agar (Hangzhou Microbial Reagent Co., Ltd.) and lysine indole motility semisolid agar (Hangzhou Microbial Reagent Co., Ltd.). Identification of serovars was performed by slide agglutination tests (Hangzhou Microbial Reagent Co., Ltd.), according to the Kauffmann-White scheme. All of the isolates were stored in 25% glycerol at -80°C until use.

Antimicrobial susceptibility testing: For the determination of antimicrobial susceptibility of the isolates, the disk diffusion methods were performed according to the Clinical and Laboratory Standards Institute (CLSI, 2006) standards. The following antimicrobials (Oxoid) were tested: amoxicillin (10 μg), ampicillin (10 μg), carbenicillin (100 μg), ceftriaxone (30 μg), cefotaxime (30 μg), gentamicin (10 μg), kanamycin (20 μg), streptomycin (10 μg), spectinomycin (100 μg), chloramphenicol (30 μg), tetracycline (30 μg), trimethoprim (5 μg), trimethoprime/sulfamethoxazole (1.25-23.75 μg), sulfafurazole (300 μg), ciprofloxacin (5 μg) and nalidixic acid (30 μg). Reference strains *E. coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were included as controls. For each isolate, the zone of inhibition around each disk was measured, after incubation at 37°C for 24 h.

RESULTS AND DISCUSSION

The percentage of *Salmonella*-positive samples was 4.8, 5.3, 10.7 and 9.9% for chicken, duck, goose and pig samples, respectively (Table 1). In Shanghai which is located in eastern China, the isolation rates of *Salmonella* sp. from faecal samples were 4.5% in 550 chickens between 2005 and 2006 (Liu *et al*., 2010).

The present results demonstrated similar *Salmonella* sp. isolation rates to this previously reported in China. The prevalence of *Salmonella* sp. in pigs in this study was lower than the 14.2% reprted by Murugkar *et al.* (2005). However, Kishima *et al.* (2008) showed that *Salmonella* sp. prevalence was 3.3% in pig faecal samples in 2004-2005. It is difficult to compare the results in other countries because there are variations in sampling methods and methods for isolation of *Salmonella* sp. (Kishima *et al*., 2008).

About 15 serovars were found among the 163 *Salmonella* isolates, including 4 serovars in chicken isolates, 6 serovars in duck isolates, 5 serovars in goose isolates and 9 serovars in pig isolates (Table 2). The predominant serovars (*S. Senftenberg*, *S. Typhimurium*, *S. Pullorum* and *S. Enteritidis*) accounted for about 75.5% of the isolates. *S. Pullorum* (84.9%) was the most common serovar among the 33 *Salmonella* isolates from chckens in this study as well as in previous studies (Liu *et al*., 2010). *S. Pullorum*, the causative agent of Pullorum disease is the most prevalent host-adapted pathogen in China (Pan *et al*., 2009). However, various researchers have documented the significance of *S. Typhimurium* (Cheong *et al*., 2007), *S. Enteritidis* (Van Duijkeren *et al*., 2002), *S. Infantis* (Ishihara *et al*., 2009) and *S. Emek* (Vo *et al*., 2006) in chickens in other countries. In duck, serovar Typhimurium, Newport and Saintpaul were predominant and represented 26.7, 20 and 20%, of the 15 duck isolates, respectively.

Ogasawara *et al*. (2008) also found *S. Typhimurium* (24%) were frequently isolated from ducks during 1999-2001 in Vietnam, but the serovar Newport and Saintpaul were not isolated. The predominant *Salmonella* serovars from duck eggs in Thailand were Typhimurium (23.3%), Cerro (17.7%) and Tennessee (12%) (Tran *et al*.., 2004). Thus, the ducks seem to be sensitive to *S. Typhimurium. S. Senftenberg* (66.7%) was the most prevalent serovar among 54 isolates originating from goose, followed by *S. Typhimurium* (18.5%) and *S. Newport* (11.1%). Few reports regarding the prevalance of *Salmonella* sp. in geese have been published. Trawinska *et al*. (2008) found that serovars Typhimurium (44.8%) and Enteritidis (29%) were predominant in the isolates of geese in 2001-2005 in Poland. Among the 61 pig isolates, *S. Enteritidis* (23%) and *S. Typhimurium* (21.3%) were the most common serovars, followed by *S. Senftenberg* (18%) and *S. Derby*

Table 2: Distribution of *Salmonella* serovars in animals of eastern China

	No. of isolates				
Serovar	Chicken	Duck	Goose	Pig	Total (%)
Senftenberg	-	1	36	11	48 (29.4)
Typhimurium	2	4	10	13	29 (17.8)
Pullorum	28 (24)[a]	-	-	-	28 (17.2)
Enteritidis	2	2	-	14 (4)[a]	18 (11.1)
Newport	-	3	6	-	9 (5.5)
Derby	-	-	1	7	8 (4.9)
Agona	-	-	-	5	5 (3.1)
Choleraesuis	-	-	-	4 (4)[a]	4 (2.5)
Rissen	-	-	-	3	3 (1.8)
Saintpaul	-	3	-	-	3 (1.8)
Heidelberg	-	-	-	2	2 (1.2)
Virchow	-	-	-	2	2 (1.2)
Tshiongwe	-	2	-	-	2 (1.2)
Dublin	1	-	-	-	1 (0.6)
Anatum	-	-	1	-	1 (0.6)
Total	33 (24)[a]	15	54	61 (8)[a]	163 (100.0)

[a]Isolates from diseased animals

Table 3: Antimicrobial resistance of *Salmonella* isolates by domestic animals in eastern China

	Number of resistant isolates (%)				
Antimicrobials	Chicken (n = 33)	Duck (n = 15)	Goose (n = 54)	Pig (n = 61)	Total (n = 163)
Amoxicillin	14 (43.8)	1 (6.67)	1 (1.85)	19 (31.10)	35 (21.6)
Ampicillin	29 (90.6)	1 (6.67)	(0.00)	40 (65.60)	70 (43.2)
Carbenicillin	29 (90.6)	5 (33.30)	33 (61.10)	39 (63.90)	106 (65.4)
Ceftriaxone	4 (12.5)	(0.00)	(0.00)	2 (3.30)	6 (3.7)
Cefotaxime	1 (3.1)	1 (6.67)	(0.00)	(0.00)	2 (1.2)
Gentamicin	1 (3.1)	(0.00)	(0.00)	12 (19.70)	13 (8.0)
Kanamycin	(0.0)	(0.00)	(0.00)	12 (19.70)	12 (7.4)
Streptomycin	18 (56.3)	1 (6.67)	9 (16.70)	34 (55.70)	62 (38.3)
Spectinomycin	9 (28.1)		10 (18.50)	19 (31.10)	38 (23.5)
Chloramphenicol	4 (12.5)	1 (6.67)	(0.00)	10 (16.40)	15 (9.3)
Tetracycline	15 (46.9)	4 (26.70)	11 (20.40)	46 (75.40)	76 (46.9)
Trimethoprim	12 (37.5)	2 (13.30)	3 (5.60)	19 (31.10)	36 (22.2)
Trimethoprim/ sulfamethoxazole	10 (31.3)	2 (13.30)	9 (16.70)	33 (54.10)	54 (33.3)
Sulfafurazole	30 (93.8)	3 (20.00)	9 (16.70)	32 (52.50)	74 (45.7)
Ciprofloxacin	3 (9.4)	(0.00)	(0.00)	1 (1.64)	4 (2.5)
Nalidixic acid	30 (93.8)	8 (53.30)	6 (11.10)	35 (57.40)	79 (48.8)

(11.5%). In several countries, *S. Typhimurium* and *S. Derby* are the predominant serovars in the isolates from pigs (Boonmar *et al*., 2008; Davies *et al*., 2004). Ogasawara *et al*. (2008) showed that the predominant serovars of pig isolates were *S. Javiana* (30.8%) and *S. Derby* (15.4%) in 1999-2001. Murugkar *et al*. (2005) also showed that *S. Enteritidis* was frequently found in 44% (11/25) of the pig isolates in 2003-2004. Thus the predominant serovars of *Salmonella* sp. found in pigs in China were similar to those observed in other countries.

Higher resistance rates were observed against carbenicillin (65.4%), nalidixic acid (48.8%), tetracycline (46.9%), sulfafurazole (45.7%), ampicillin (43.2%), streptomycin (38.3%) and trimethoprim/sulfamethoxazole (33.3%). Resistance rates to ceftriaxone, ciprofloxacin and cefotaxime were <5% (Table 3). Chicken and pig are two important food animals in China and >90% of isolates in chicken were resistant to ampicillin, carbenicillin, sulfafurazole and nalidixic acid and higher than the results for *Salmonella* sp. isolated from chickens in USA (Zhao *et al*., 2007).

The prevalence of resistance of pig isolates to each of the antimicrobials ampicillin, carbenicillin, sulfafurazole, nalidixic acid, tetracycline, streptomycin and trimethoprim/sulfamethoxazole was high and these prevalences ranged approximately from 50-80%. A recent study reported that 38 *Salmonella* sp. isolates from pork samples obtained in China were resistant to tetracycline (53%), sulfafurazole (61%), ampicillin (26%), trimethoprim/sulfamethoxazole (47%), streptomycin (32%) and nalidixic acid (29%) (Yang *et al*., 2010). Chicken *Salmonella* sp. isolates displayed the highest rate of resistance to at least one antimicrobial (100%), followed

Table 4: Multidrug resistance observed among *Salmonella* isolates obtained from animals

	Percentage of resistant to indicated number of antimicrobials				
Animal species (No. isolates)	1-3	4-6	7-9	>9	Total (≥1)
Chicken (33)	6.1	54.5	21.2	18.2	100.0
Duck (15)	60.0	13.3	6.7	0.0	80.0
Goose (54)	61.1	27.8	1.8	0.0	90.7
Pig (61)	24.7	22.9	22.9	22.9	93.4
Total (163)	36.2	30.1	14.1	12.3	92.6

by those recovered from pig (93.4%), goose (90.7%) and duck (80%) (Table 4). Over three-quarter of the chicken isolates (75.7%), 45.8% of pig isolates, 29.6% of goose isolates and 20% of duck isolates showed resistance to 4-9 antimicrobials (Table 4).

Furthermore, approximately 23% of pig isolates and 18% of chicken isolates were resistant to >9 antimicrobials. A high percentage of multiple antimicrobial resistant *Salmonella* sp. from various food animals was also reported by other researchers (Ogasawara *et al*., 2008; Zhao *et al*., 2007).

As animals are a main reservoir of *Salmonella* and the use of antimicrobials in food animals for therapy, prophylaxis and growth promotion accelerates the emergence of antimicrobial resistant pathogens, it is not surprising that an increased number of human salmonellosis cases are caused by foodborne antimicrobial resistant *Salmonella* (Foley and Lynne, 2008).

In the present study, the prevalence of *S. Enteritidis*, *S. Pullorum* and *S. Typhimurium* resistance to >9 antimicrobials was 55.6, 17.9 and 17.2%, respectively (Table 5) which was in agreement with previous observations among *Salmonella* isolated from retail meats

Table 5: Multidrug resistance observed among *Salmonella* serovars obtained from animals

Animal species (No. isolates)	Percentage of resistant to indicated number of antimicrobials				
	1-3	4-6	7-9	>9	Total (≥1)
Pullorum (28)	7.1	57.1	17.9	17.9	100.0
Enteritidis (18)	22.2	0.0	22.2	55.6	100.0
Typhimurium (29)	10.3	41.4	24.1	17.2	100.0
Dublin (1)	0.0	0.0	100.0	0.0	100.0
Senftenberg (48)	54.2	27.1	0.0	0.0	81.3
Newport (9)	100.0	0.0	0.0	0.0	100.0
Anatum (1)	100.0	0.0	0.0	0.0	100.0
Derby (8)	75.0	12.5	12.5	0.0	100.0
Choleraesuis (4)	0.0	0.0	100.0	0.0	100.0
Agona (5)	0.0	100.0	0.0	0.0	100.0
Heidelberg (2)	50.0	0.0	50.0	0.0	100.0
Rissen (3)	100.0	0.0	0.0	0.0	100.0
Virchow (2)	0.0	100.0	0.0	0.0	100.0
Saintpaul (3)	100.0	0.0	0.0	0.0	100.0
Tshiongwe (2)	100.0	0.0	0.0	0.0	100.0

and food animals (Pan *et al*., 2009; Yang *et al*., 2010) but differed from some other reports (Liu *et al*., 2010; Zhao *et al*., 2007).

CONCLUSION

In conclusion, the high prevalence of *Salmonella* in domestic animals in eastern China and the level of antimicrobial resistance has raised the concerns and constituted a real threat to public health. More detailed epidemiological studies including molecular characterization would be of great interest to allow better control of this pathogen.

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