



# Occurrence, antimicrobial resistance and biofilm formation of *Salmonella* isolates from a chicken slaughter plant in China



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## ABSTRACT

An investigation was undertaken to determine the occurrence, antibiotic resistance and biofilm formation of *Salmonella* spp. isolated from a chicken slaughter plant in Anhui, China. A total of 104 samples (52 from chicken carcasses and 52 from processing contact-surfaces) were collected from three processing points in a chicken slaughterhouse. The 23 isolates (22.1%, 23/104) were confirmed for *Salmonella* and belonged to six different serotypes, including *S. Indiana* ( $n = 9$ ), *S. Infantis* ( $n = 4$ ), *S. Derby* ( $n = 3$ ), *S. Heidelberg* ( $n = 2$ ), *S. Agona* ( $n = 2$ ) and *S. Typhimurium* ( $n = 1$ ), whereas two isolates ( $n = 2$ ) were non-typable. Significant differences in occurrence were found between post-evisceration, post-chilling and post-grading processing points. A total of 20 (87%, 20/23) isolates were resistant to at least one antibiotic, of which 19 isolates (95%, 19/20) showed 13 multiple antibiotic resistance patterns against 11 different antibiotics. Resistance to ampicillin (78.3%, 18/23) was the most common. The multiple antibiotic resistance (MAR) index varied from 0.27 to 0.91. The *Salmonella* isolates from the chicken plant and from humans in the same area who were suffering *Salmonella* infections showed similar antimicrobial resistance patterns, namely resistance to ampicillin, trimethoprim/sulfamethoxazole, gentamicin, chloramphenicol and tetracycline, and abbreviated as “ATsGCT”. Meanwhile, *Salmonella* isolates exhibited variation in biofilm-forming behavior with regards to the incubation media and serotypes, a relatively high biofilm production was observed for *S. Agona* incubated in MTLB (meat thawing-loss broth) at 72 h. There was no significant correlation between antimicrobial resistance and biofilm formation of isolates. Our findings provide baseline information on the distribution of *Salmonella* serovars in this plant, and provide support to the need for improved farming practice and for more prudent use of antimicrobial agents.

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## 1. Introduction

*Salmonella* spp. is a globally widespread food-borne pathogen, and its outbreaks are commonly associated with the consumption of contaminated food such as eggs, poultry meat and pork. In EU, *Salmonella* has become the major cause of food poisoning (EFSA-ECDC, 2012a), while in the USA, contaminated food with *Salmonella* has resulted in numerous recalls and outbreaks during 2012 that have been addressed by the U.S. Department of Agriculture and Center for Disease Control and Prevention ([www.fda.gov/Safety/Recalls](http://www.fda.gov/Safety/Recalls)).

Recently, much research interest has been drawn to the high occurrence, and antimicrobial resistance of *Salmonella* in livestock for food products, which may have the potential to transmit to humans through the food chain. Epidemiological studies have

implicated foods of animal origin as the major vehicles associated with illnesses caused by *Salmonella* (Dallal et al., 2010). Despite the occurrence of *Salmonella* in raw and retail cooked meats have previously been reported (Cook, Odumeru, Lee, & Pollari, 2012; Fearnley, Raupach Lagala, & Cameron, 2011), information on the occurrence of *Salmonella* at slaughter facilities is limited, even though it is considered as the main source of cross-contamination. Investigation of occurrence of *Salmonella* in slaughter plants may aid in optimizing HACCP strategy and ensure meat safety in China. Furthermore, investigation of the occurrence and distribution of *Salmonella* at different slaughter processing stations would be of benefit for the study of the quantitative exposure assessment of *Salmonella* in chicken meat.

Emergence of multiple resistance of *Salmonella* isolates and special antimicrobial resistance patterns have raised increasing concern among governments all over the world (Chao, Zhou, Jiao, Qian, & Xu, 2007; EFSA-ECDC, 2012b; Thong & Modarressi, 2011). The link between antimicrobial resistance of *Salmonella* isolates and

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outbreaks of human poisoning has already been demonstrated (Folster et al., 2012). In China, human foodborne illnesses have been associated with the antimicrobial resistance of *Salmonella* isolates (Ran et al., 2011; Yu, Chen, Yu, Li, et al., 2011; Yu, Chen, Yu, Pan, et al., 2011), especially the antibiotic-resistant *Salmonella* Typhimurium and *Salmonella* Enteritidis have caused human suffering from serious diarrhoea, the general resistance antibiotics included chloramphenicol, ampicillin, tetracycline, sulfamethoxazole, gentamicin etc., and the common multiple resistance patterns were ACTsT type (resistance to at least ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole, and tetracycline). Therefore, monitoring of *Salmonella* occurrence and its antimicrobial resistance in the meat-food supply, especially for raw meat in the slaughter plant, is necessary because of the public health implications of a potential spread of antimicrobial resistant *Salmonella*.

*Salmonella* can grow as surface-associated aggregates on food contact-surfaces and equipment (Chia, Goulter, McMeekin, Dykes, & Fegan, 2009), commonly referred to as biofilms. The cells in biofilms are potential sources of contamination of food products. Biofilms may play a crucial role in the survival of *Salmonella* under unfavorable environmental conditions, such as in animal slaughterhouses and processing plants. To date, relatively little research has examined the ability of biofilm formation of *Salmonella* isolated from slaughter plants under the conditions commonly encountered. Moreover, the relationship between antimicrobial resistance and the ability of *Salmonella* isolates to form biofilms is unknown. The present study was therefore carried out to (i) determine the occurrence and distribution of *Salmonella* at processing points at a chicken slaughterhouse, (ii) identify the antimicrobial resistance patterns and the ability of biofilm formation of *Salmonella* isolates, and (iii) evaluate the correlation between number of isolates resistant to antibiotics and biofilm formation of *Salmonella* isolates.

## 2. Materials and methods

### 2.1. Sample collection

A total of 104 samples, collected from chicken carcass surfaces ( $n = 52$ ) and processing contact-surfaces ( $n = 52$ ), were obtained from three processing points (post-evisceration point  $n = 40$ , post-chilling point  $n = 32$  and post-grading point  $n = 32$ ) at a chicken slaughter plant in Anhui Province (China), during September 2011 (Fig. 1). The samples were collected on each Wednesday of three consecutive weeks (35, 35 and 34 samples at each time, respectively). At each collection point, the number of the samples from carcass surfaces was the same as that of the samples from processing surfaces. The sample collection were performed according to the standard methods recommended by USDA-FSIS, which involved rinsing the whole chicken carcass with 400 mL buffered peptone water (BPW) and swabbing the contacted-surfaces (including evisceration equipment, transport belts and cutting

boards) with swabs moistened with 0.1% BPW (Adzitey, Rusul, & Huda, 2012). BPW was used as the pre-enrichment broth.

### 2.2. Identification of isolates

*Salmonella* isolates were identified according to the China national food safety standard methods – Food microbiological examination: *Salmonella* (GB 4789.4-2010). Tetrathionate broth base (TTB) and selenite cystine (SC) broth were used as the selective enrichment broth, and xylose lysine desoxycholate agar (XLD) and bismuth sulfite agar (BS) were used as the plating media. All culture media were obtained from Luqiao (Luqiao Technology Co. Ltd., China). Isolates were further identified by colony morphology, biochemical characterization and special virulence genes (*invA* and *hlyA*) of *Salmonella*, the two virulence genes were detected by PCR (primers of *invA*: F-tcg tcac tcc att acc tac c, R-aaa cgt tga aaa act gag ga; primers of *hlyA*: F-cgc cgg cga gat tgt gag ta, R-aga ctc tcg gat tga acc tga) (Jacobsen & Holben, 2007; McCabe, Burgess, O'Regan, et al., 2011). Isolates were then serotyped with specific O and H *Salmonella* antisera, and classified according to the Kauffman–White scheme.

### 2.3. Screening for antimicrobial resistance

Antimicrobial susceptibility testing was determined by the disk diffusion method on Mueller-Hinton agar, using 11 different antibiotics belonging to 7 different classes. The antibiotics used were as follows, penicillins (ampicillin, 10 µg), cephalosporins (cefalothin, 30 µg; cefoxitin, 30 µg; ceftriaxone, 30 µg), aminoglycosides (amikacin, 30 µg; gentamicin, 30 µg), tetracyclines (tetracycline, 30 µg), quinolones (ciprofloxacin, 5 µg; enrofloxacin, 5 µg), amphenicols (chloramphenicol, 5 µg), sulfonamides (compound sulfamethoxazole (trimethoprim/sulfamethoxazole), 1.25/23.75 µg). The results were interpreted according to the standard of “Performance standards for antimicrobial susceptibility testing” recommended by Clinical and Laboratory Standard Institute (CLSI) of USA. The strain *Escherichia coli* ATCC 25922 was used as quality control strain. The antimicrobial resistance of each isolate was performed with three repetitions. The multiple antibiotic resistance (MAR) index was calculated with previous method (Adzitey et al., 2012) and by employing the following formula: MAR index = (Number of resistance antibiotics per isolate)/(total number of antibiotics tested). Isolates classified as intermediate on the basis of the inhibition zone were considered as sensitive for the MAR index (Singh, Yadav, Singh, & Bharti, 2010).

### 2.4. Preparation of inocula

Isolates were individually cultured and sub-cultured (37 °C, 24 h) in tryptic soytone broth (TSB). Cells were centrifuged, and cell pellets were washed three times with phosphate buffered saline (PBS, pH 7.2). Each pellet was resuspended in PBS and adjusted to a final concentration of  $10^8$  CFU/mL. In order to simulate contamination of surfaces in meat processing plants, chicken meat thawing-loss broth (MTLB) was used as a culture medium. It was prepared as described previously (Midelet & Carpentier, 2002). The protein content of MTLB was adjusted to a final concentration of 5 mg/mL using the Biuret protein assay.

### 2.5. Biofilm assay

An aliquot (20 µL,  $10^7$  CFU/mL) of each isolated culture was transferred to 180 µL of fresh TSB or MTLB in a 96-well polystyrene microplate. The microplates were then incubated at 18 °C and the biofilm was assayed at 24 h and 72 h. The two broths, devoid of

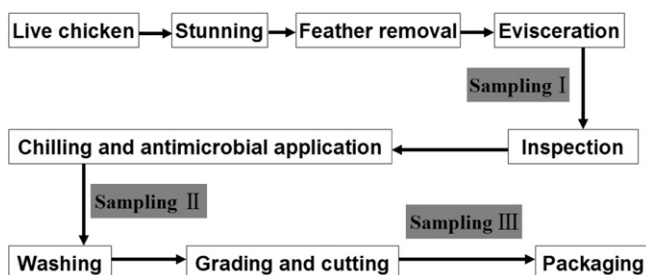


Fig. 1. The slaughter processing of chicken in plant and sampling points (gray pane).

bacterial inocula, served as negative controls. Following incubation, wells were rinsed three times with sterile de-ionized water and the plates were air-dried for 45 min, and then each well was stained with 200  $\mu$ L of 0.25% (wt/vol) crystal violet for 30 min (Kim & Wei, 2009). The staining solution was then removed, and the wells were rinsed three times with sterile de-ionized water. The crystal violet bound to the biofilm was then solubilized with 200  $\mu$ L of 95% ethanol for 30 min. The absorbance was measured at 570 nm (Silagyi, Kim, Lo, & Wei, 2009).

## 2.6. Statistical analysis

The chi-square test was performed to compare the occurrence of *Salmonella* spp. between different processing points. Pearson's and Spearman's correlations between number of isolates resistant to antibiotics and amount of biofilm formation was determined using SPSS 13.0 (SPSS Inc, Chicago, IL, USA). In all cases, the level of statistical significance was  $p < 0.05$ .

## 3. Results

### 3.1. The occurrence of *Salmonella* spp.

Of the samples tested, 22% (23/104) were positive for *Salmonella*. In addition to identification of the isolates by biochemical characterization, we further identified them by detecting *invA* and *hilA* genes, which are commonly used as the PCR diagnostic targets for *Salmonella* in the food industry and research fields (Joshi et al., 2009; McCabe, Burgess, Walsh, et al., 2011). The results (Table 1) indicated that all the 23 isolates were positive for detection of *invA* and *hilA* genes. These isolates were serotyped into 6 serovars (Table 1), including *S. Indiana* ( $n = 9$ ), *S. Derby* ( $n = 3$ ), *S. Heidelberg* ( $n = 2$ ), *S. Agona* ( $n = 2$ ), *S. Infantis* ( $n = 4$ ), *S. Typhimurium* ( $n = 1$ ). However, two isolates ( $n = 2$ ) obtained from processing contact-surfaces which were confirmed for *Salmonella* spp. by biochemical characterization and detection of virulence genes were non-typable. 19% (10/52) and 25% (13/52) of *Salmonella* were identified from chicken carcasses and from contact-surfaces, respectively. *S. Heidelberg* and *S. Typhimurium* were identified only in samples from contact-surfaces (Table 1).

The presence of *Salmonella* in post-evisceration, post-chilling and post-grading processing points are presented in Fig. 2. There was significant variation ( $p < 0.05$ ) in the occurrence of *Salmonella* at the different processing points, where incidence levels decreased with progress through the plant. Compared to the occurrence at the

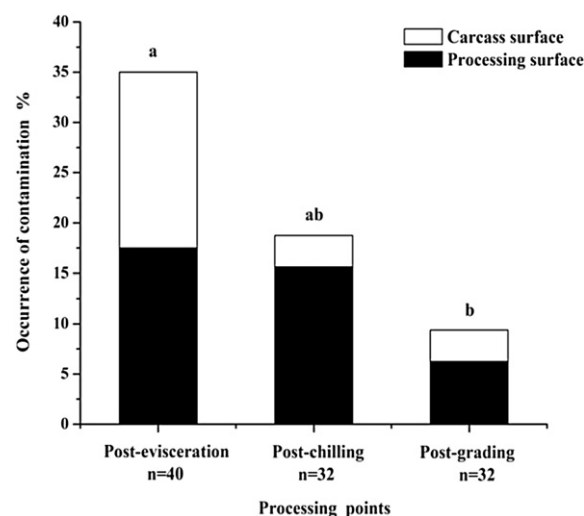


Fig. 2. Occurrence of contamination of *Salmonella* spp. Chi-square test (Fisher) was performed on samples at each processing point, values with different lower-case letters (a and b) at each processing point are significant different ( $p < 0.05$ ).

post-evisceration point (35%), the occurrence of *Salmonella* significantly ( $p < 0.05$ ) decreased at post-chilling (19%) and post-grading (9%) points. However, compared to the decrease of occurrence at post-chilling point associating with the low occurrence of carcasses, the decrease of occurrence at post-grading point mainly resulted from the low occurrence of processing surfaces.

### 3.2. Antimicrobial resistance

Of 23 isolates, 78% of isolates were resistant to ampicillin (Table 2). 13% were resistant to cefoxitin and ciprofloxacin, respectively. In addition, 74% of isolates was resistant to cefalothin, 61% to ceftriaxone, 74% to compound sulfamethoxazole, 39% to amikacin, 48% to gentamicin, 61% to chloramphenicol, 39% to enrofloxacin and 70% to tetracycline (Table 2). There were great differences in antimicrobial resistance for each of the *Salmonella* serovars (Table 2). It was found that *S. Indiana*, *S. Typhimurium* and *S. Heidelberg* showed the highest percentage of resistance, followed by *S. Agona* and *S. Derby*. Three *S. Infantis* isolates (C6, C8 and C19) were susceptible to all tested antibiotics (Table 3).

Of 23 isolates, 20 showed resistance to antibiotics, 19 of which showed multiple resistances. One isolate (C23, belonging to

Table 1  
Sources and serotypes of *Salmonella* spp. isolates.

Isolates	Sources	Sampling area	Serotypes	<i>InvA</i>	<i>hilA</i>	Isolates	Sources	Sampling area	Serotypes	<i>InvA</i>	<i>hilA</i>
C1	CS	PC	Indiana (9)	+	+	C9	PS	PE	Heidelberg (2)	+	+
C2	PS	PC		+	+	C10	PS	PC	Agona (2)	+	+
C5	PS	PC		+	+	C4	CS	PE		+	+
C12	PS	PE		+	+	C11	PS	PC	Infantis (4)	+	+
C13	CS	PE		+	+	C6	CS	PE		+	+
C15	PS	PE		+	+	C8	CS	PE		+	+
C16	CS	PE		+	+	C19	PS	PG		+	+
C18	PS	PE	Derby (3)	+	+	C23	CS	PG	Typhimurium (1)	+	+
C21	CS	PE		+	+	C17	PS	PE		+	+
C3	CS	PC		+	+	C14	PS	PE		+	+
C7	CS	PE		+	+	C20	PS	PE	Unknown (2)	+	+
C22	PS	PG		+	+						

CS, carcass surfaces; PS, processing contact-surfaces; +, positive detection of gene by PCR. PE, post-evisceration point; PC, post-chilling point; PG, post-grading point.

**Table 2**Percentage *Salmonella* serovars resistant to different antibiotics.

Serotypes	Percentage of each serotype resistant to each antibiotic (%)											Overall (%)
	AMP	CEF	FOX	CRO	AMK	GEN	TET	CIP	ENX	CHL	COSMZ	
Indiana	100 (9/9) <sup>a</sup>	100 (9/9)	11 (1/9)	78 (7/9)	67 (6/9)	67 (6/9)	89 (8/9)	0 (0/9)	56 (5/9)	89 (8/9)	89 (8/9)	67 (67/99)
Derby	67 (2/3)	33 (1/3)	0 (0/3)	0 (0/3)	0.0 (0/3)	0 (0/3)	100 (3/3)	0 (0/3)	0 (0/3)	33 (1/3)	67 (2/3)	27 (9/33)
Heidelberg	100 (2/2)	100 (2/2)	0 (0/2)	100 (2/2)	50 (1/2)	100 (2/2)	100 (2/2)	0 (0/2)	50 (1/2)	100 (2/2)	100 (2/2)	73 (16/22)
Agona	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)	50 (1/2)	41 (9/22)
Infantis	0 (0/4)	0 (0/4)	0.0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	25 (1/4)	2 (1/44)
Typhimurium	100 (1/1)	100 (1/1)	0.0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	82 (9/11)
Non-typable	100 (2/2)	100 (2/2)	0 (0/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	91 (20/22)
Overall (%)	78 (18/23)	74 (17/23)	13 (3/23)	61 (14/23)	39 (9/23)	48 (11/23)	70 (16/23)	13 (3/23)	39 (9/23)	61 (14/23)	74 (17/23)	

AMP, ampicillin; CEF, cefalothin; FOX, cefoxitin; CRO, ceftriaxone; AMK, amikacin; GEN, gentamicin; TET, tetracycline; CIP, ciprofloxacin; ENX, enrofloxacin; CHL, chloramphenicol; COSMZ, compound sulfamethoxazole.

<sup>a</sup> No. of isolates resistant to a particular antibiotic.

S. Infantis) was only resistant to compound sulfamethoxazole, and 13 different patterns of resistance were observed (Table 3). It was observed that isolates from processing surfaces showed resistance to as many as 10 antibiotics, in comparison to isolates from carcass surfaces, which were resistant to 1–5 antibiotics, except for C1 (belonging to S. Indiana) isolate which was resistant to 8 antibiotics. The maximum and average multiple antibiotic resistance (MAR) indices of isolates were 0.91 and 0.52, respectively, and the MAR index of other isolates ranged from 0.27 to 0.82.

### 3.3. Biofilm formation

The amounts of biofilm formation of *Salmonella* isolates were significantly influenced by the types of growth media and incubation time (data not shown). Biofilm formation of these isolates was serotype dependent. Relatively high amounts of biofilm were produced by S. Agona (C4), S. Infantis (C6 and C19), S. Indiana (C13 and C16) isolates.

### 3.4. Correlation between number of isolates resistance to antibiotics and the ability to produce biofilm

A preliminary correlation analysis was carried out to determine whether any relationship existed between the number of isolates resistance to antibiotics and the ability of *Salmonella* isolates to form biofilm. The Pearson's and Spearman's correlation coefficients were shown in Table 4. The findings suggested that no significant correlation ( $p > 0.05$ ) existed between biofilm formation and the number of antimicrobial resistant isolates.

## 4. Discussion

Poultry foods are generally considered to be at a higher risk for *Salmonella* contamination than other foods. In 2012, several outbreaks of *Salmonella* have been associated with poultry meat and products ([www.cdc.gov/salmonella/outbreaks.html](http://www.cdc.gov/salmonella/outbreaks.html)). During chicken slaughter, *Salmonella* from the gastro-intestinal tract of chickens carrying pathogens can contaminate the carcasses and the processing line (Rostagno, Wesley, Trampel, & Hurd, 2006). Although efforts at controlling *Salmonella* have concentrated primarily on controlling contamination within the slaughterhouse, a high proportion of carcasses are still found to be contaminated with *Salmonella*. In our survey, we identified six different serotypes of *Salmonella* (23 isolates), S. Indiana was the common serotype, followed by S. Infantis. As similar to our observation, a survey conducted by Yang et al. (2010) in China also found that S. Indiana was the common serotype, followed by S. Infantis. In our study, the occurrence of *Salmonella* from processing surfaces (25%, 13/52) was higher than from chicken carcasses (19%, 10/52). A survey conducted by Adzitey et al. (2012) showed a similar incidence of surface contamination for *Salmonella* (23.5%) in a poultry processing environment. However, a higher occurrence was observed in another study (Antunes, Réu, Sousa, Peixe, & Pestana, 2003), where 60% of poultry meat was contaminated with *Salmonella*. The high occurrence in poultry meat products indicates that the production systems and processing environments of poultry meat may facilitate the presence of *Salmonella* in the final products. In our findings (Fig. 2), the occurrence significantly decreased at the post-grading point, however, there were still 3% *Salmonella* of the carcasses

**Table 3**Multiple antibiotic resistance patterns of *Salmonella* isolates.

Serotypes and isolates	No. of antibiotics	Multiple antibiotic resistance pattern	No. (%) of isolates	MAR index
S. Infantis (C8, C6 and C19)	0	Susceptible to all tested antibiotics	3 (13.04)	0
S. Infantis (C23)	1	COSMZ	1 (4.35)	0.09
S. Indiana (C21)	3	AMP + CEF + FOX	4 (17.39)	0.27
S. Derby (C7)		TET + COSMZ + CHL		
S. Derby (C22)		TET + COSMZ + AMP		
S. Derby (C3)		TET + AMP + CEF		
S. Agona (C4)	4	CRO + AMP + CEF + FOX	1 (4.35)	0.36
S. Agona (C11)	5	CRO + AMP + COSMZ + CEF + FOX	2 (8.70)	0.45
S. Indiana (C13)		TET + AMP + COSMZ + CEF + CHL		
S. Heidelberg (C10)	8	TET + CRO + AMK + AMP + COSMZ + CEF + GEN + CHL	6 (26.09)	0.73
S. Indiana (C5 and C16)				
S. Indiana (C10)		AMK + ENX + TET + CRO + AMP + COSMZ + CEF + CHL		
S. Heidelberg (C9)		TET + ENX + CRO + AMP + COSMZ + CEF + GEN + CHL		
S. Indiana (C2)				
S. Indiana (C12, C18 and C15)	9	TET + ENX + CRO + AMK + AMP + COSMZ + CEF + GEN + CHL	4 (17.39)	0.82
S. Typhimurium (C17)		CIP + TET + ENX + CRO + AMP + COSMZ + CEF + GEN + CHL		
Serotype unknown (C14 and C20)	10	CIP + TET + ENX + CRO + AMP + AMP + COSMZ + CEF + GEN + CHL	2 (8.70)	0.91

AMP, ampicillin; CEF, cefalothin; FOX, cefoxitin; CRO, ceftriaxone; AMK, amikacin; GEN, gentamicin; TET, tetracycline; CIP, ciprofloxacin; ENX, enrofloxacin; CHL, chloramphenicol; COSMZ, compound sulfamethoxazole.



**Table 4**

Pearson's and Spearman's correlations between number of isolates resistant to antibiotics and amount of biofilm formation during incubation at 18 °C for all isolates.

	Biofilm formation			
	24 h-TSB	72 h-TSB	24 h-MTLB	72 h-MTLB
No. of isolates resistant to antibiotics	<sup>a</sup> 0.082	<sup>a</sup> 0.191	<sup>a</sup> 0.081	<sup>a</sup> 0.203
24 h-TSB		<sup>b</sup> 0.453*	<sup>b</sup> 0.805**	<sup>b</sup> 0.183
72 h-TSB			<sup>b</sup> 0.554**	<sup>b</sup> 0.715**
24 h-MTLB				<sup>b</sup> 0.406

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; 24 h or 48 h-TSB or MTLB indicated that the biofilm was incubated in TSB or meat thawing-loss broth (MTLB) for 24 h or 72 h.

<sup>a</sup> Spearman correlation.

<sup>b</sup> Pearson's correlation.

positive in the final products at the post-grading point (Fig. 2), which was the last stage before packaging the product. This is not acceptable according to the food safety standard of China (NY 5034-2005), and the residual *Salmonella* may cause serious cross-contamination during the subsequent retail process and cause serious risk to public health. The presence of *Salmonella* on products and contacted-surface indicated that appropriate hygienic measures should be applied to control *Salmonella* in this slaughter plant.

In our study, 87% (20/23) of the isolates were resistance to at least one of the antibiotic tested, and 95% (19/20) of the isolates showed multiple resistance (Table 3). The most common serotype (*S. Indiana*) showed multi-drug resistant phenotypes, which also appear to be widely available in China (Lu et al., 2011). Multidrug resistant phenotypes also emerged in another four serotypes (*S. Derby*, *S. Heidelberg*, *S. Agona* and *S. Typhimurium*). The presence of multiple-resistant isolates in meat may cause serious human poisoning, the isolates may be transferred from meat to humans, not only through direct contact but also indirectly. This indirect transfer involves mainly consumption of meat or other food contaminated with *Salmonella* (Depoorter et al., 2012), because antibiotic resistance genes frequently are located on mobile genetic elements. Horizontal gene transfer between food bacteria and human intestinal bacteria has been demonstrated, not only *in vitro*, but also *in vivo* (Dahl et al., 2007; Smet et al., 2010). The hypothesis of transfer of resistant *Salmonella* from livestock, via the consumption of meat to humans, has been confirmed, and the link between antimicrobial resistant *S. Typhimurium* from pork and outbreaks of human poisoning has already been demonstrated (Van Boxtael et al., 2012). In our study, 14 isolates of multiple-resistant *Salmonella* were resistant to 5–10 antibiotics, and showed 8 types of resistance patterns, with the maximum MAR index of isolates being 0.91 (Table 3). Similar serotype and multidrug resistant patterns of *Salmonella* isolate from human foodborne illness have been identified in surrounding areas of this slaughter plant (Ran et al., 2011; Yu, Chen, Yu, Li, et al., 2011). Notably, the *S. Typhimurium* identified in our study showed the multiple resistance patterns quietly similar to the *S. Typhimurium* isolates found in human foodborne illness in surrounding areas, especially the multiple resistance patterns of ATsGCT type (resistance to at least ampicillin, trimethoprim/sulfamethoxazole, gentamicin, chloramphenicol and tetracycline), which has been associated with serious diarrhoea (Yu, Chen, Yu, Pan, et al., 2011). There may be some relationship between the chicken meat contaminated by *Salmonella* in this plant and the diarrhoea infection in surrounding areas, because the plant, which was a large-scale chicken slaughtering and processing plant, supplied the vast majority of chicken meat consumption to consumers in surrounding areas. The contaminated chicken meat or other food contact with the chicken meat may be the source of infection.

Ampicillin resistance has been observed amongst the *Salmonella* isolates from poultry products (Adzitey et al., 2012; Antunes et al., 2003). In our study, a total of 78% of the *Salmonella* isolates were

found to be resistant to ampicillin (Table 2), but this was much lower than that reported in isolates from foods from Poland where 93% were ampicillin-resistant (Wasył & Hoszowski, 2012). This high antimicrobial resistance may result from extensive use or misuse of penicillins, which has been used for therapeutics of infectious diseases in animal feeds. Cephalosporins are usually grouped into “generations” by their antimicrobial properties. In our study, we found that 74%, 13% and 61% isolates were resistance to cefalothin (first generation), ceftiofur (second generation) and ceftriaxone (third generation), respectively (Table 2). Compared to resistance to ceftriaxone, we found a lower incidence of resistance to ceftiofur; this may be caused by less use of this second generation cephalosporin in therapeutics of infections, because the third generation cephalosporin drugs were the common choice in treating life-threatening systemic infection of animals in China (Lei et al., 2010).

Two antibiotics of the aminoglycoside class were assayed in the present study. Unlike the high incidence of resistance to amikacin (39%) and gentamicin (47%) in our study, a very low incidence (<2.5%) of resistance was observed by Chao et al. (2007). High resistance to tetracyclines (70%) was observed in our study. Tetracyclines are not only used as treatments in veterinary medicine, but also as a growth enhancer in poultry production. This practice has greatly promoted the occurrence of antimicrobial-resistant isolates. Compared to our study (70%), a higher incidence (100%) of resistance to tetracyclines was reported by Glenn et al. (2011), however, a lower incidence (13%) was observed by Mezali and Hamdi (2012) in meat and meat products isolates.

In this study, the isolates showed resistance to two antibiotics from the quinolones class, namely ciprofloxacin (13%) and enrofloxacin (40%). The low incidence of resistance to ciprofloxacin has also been observed in other studies. Only 3% of *Salmonella* isolated from the retail meats was resistance to ciprofloxacin according to a survey in Malaysia (Chia et al., 2009), and 100% of *Salmonella* isolates isolated from meat were susceptible to ciprofloxacin (Singh, Agarwal, Tiwari, & Singh, 2012). Although the resistance of quinolone in *Salmonella* remains low, outbreaks of quinolone-resistant *Salmonella* infections have been reported in the United States (CDC, 2010). In our study, we found high incidences of resistance against chloramphenicol, a class of amphenicols (61%), and the compound sulfamethoxazole, a class of sulfonamides (74%). The two antibiotics are commonly used as veterinary medicines in livestock production. Recently, an increasing occurrence of resistance to the two antibiotics have already been demonstrated in many countries (Glenn et al., 2011; Iossifidou, Abraham, Soutos, Triantafyllou, & Koidis, 2012; Marrero-Ortiz et al., 2012; Van Boxtael et al., 2012). The appearance of antimicrobial resistant *Salmonella* isolates may result in serious human and livestock infections, and effective strategies and new legislation must be established to ensure a decrease in the appearance of antimicrobial resistant isolates.

Recently, attachment and biofilm formation of food-borne pathogens has become one of the central areas of food safety research because of the likelihood of potential cross-contamination and serious food safety problems. Previous studies have demonstrated that there was a correlation between biofilm formation *in vitro* using polystyrene micro-wells and biofilm formation on several surfaces commonly encountered in food facilities (Patel & Sharma, 2010; Vestby, Møretro, Langsrud, Heir, & Nesse, 2009). So, we evaluated the ability of biofilm formation of *Salmonella* isolates by polystyrene micro-wells methods, which allows large numbers of isolates under different conditions to be studied at the same time. In our study, a meat-based medium (MTLB) which is equivalent to that likely to be found in meat processing facilities and a laboratory medium (TSB, optimal for *Salmonella* growth) were tested at 18 °C for their influence on biofilm formation of

*Salmonella* isolates. The temperature of 18 °C was selected as it is a common temperature found in meat processing facilities in China. Using meat-based medium as a substrate for assessing the *Salmonella* attachment may be useful for obtaining more realistic results than the use of a specialized laboratory medium when considering the nutrient availability in foods and their interactions with the surrounded environment. High inter-isolate variation in behavior of the formation of *Salmonella* biofilm was observed with regards to the incubation media and incubation time, and this is consistent with previous reports (Díez-García, Capita, & Alonso-Calleja, 2012; Saa, Cabo, & Rodríguez, 2009). Compared to the incubation in TSB, most of the isolates showed large differences in biofilm formation incubated in MTLB. Our results provided some information for further supporting the contention that studying biofilm formation using a laboratory medium (TSB) has limited significance to the understanding of biofilm formation under the processing and handling conditions that are present in the meat industry. The biofilm formation in our study indicated that *Salmonella* isolates had the potential for attachment on contact-surfaces in a meat processing environment. The attachment *Salmonella* may result in serious problems for the meat industry.

We found there was no correlation between them (Table 4). Such a relationship has been described for other bacteria, although the findings were sometimes inconsistent and the correlations were species-dependent. Chen et al. (2010) showed that there was no correlation between biofilm-forming ability and drug resistance patterns of *E. coli*. However, another study demonstrated that there was a significant positive correlation between antibiotics resistance and biofilm-forming ability of *Pseudomonas aeruginosa* (Fricks-Lima et al., 2011).

In conclusion, this study demonstrated that the occurrence of *Salmonella* in a chicken processing plant was relatively high, a total of 23 isolates of *Salmonella* belonging to 6 serotypes were identified, including *S. Indiana*, *S. Derby*, *S. Heidelberg*, *S. Agona*, *S. Infantis* and *S. Typhimurium*. All isolates showed 13 multiple antibiotic resistance patterns against 11 different antibiotics. The percentages of antibiotic resistance ranged from 13% to 78%, and the MAR index varied from 0.09 to 0.91. Meanwhile, our results showed that the amounts of biofilm were generally related to the specific isolates and incubation media. Although samples were obtained from only one processing plant, the results provide useful information regarding the extent of contamination by *Salmonella* and serovars in raw chicken and their ability to persist in the processing environment and resist antibiotics. This has significant implications for the food safety and public health of consumers in China or other countries which may import the product. Clearly, further studies with additional isolates originating from both chicken meat products and infected humans need to be conducted in order to reveal the epidemiological characteristics of *Salmonella*, and the relationship between *Salmonella* isolates from chicken meat and outbreaks of human salmonellosis.

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