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Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China

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ABSTRACT

A total of 764 retail meat including 515 chicken, 91 pork, 78 beef and 80 lamb samples were collected in Shaanxi Province of China in 2007–2008 to determine the prevalence of *Salmonella*. The isolates were characterized using serotyping, antimicrobial susceptibility testing, and the presence of bla_{CMY-2} and bla_{TEM} and class I integrons. Selective serovars were further subtyped using PFGE. Approximately 54% (276) of chicken, 31% (28) of pork, 17% (13) of beef and 20% (16) of lamb samples were positive of *Salmonella*. Among 24 serovars identified, Enteritidis (31.5%) was most common, followed by Typhimurium (13.4%), Shubra (10.0%), Indiana (9.7%), Derby (9.5%) and Djugu (7.0%). Nearly 80% of the isolates (283) were resistant to at least one antimicrobial, and 53% (191) to more than three antimicrobials. Resistance was most frequently observed to sulfamethoxazole (67%), to trimethoprim/sulfamethoxazole (58%) and to tetracycline (56%). Furthermore, many isolates were resistant to nalidixic acid (35%), ciprofloxacin (21%) and ceftriaxone (16%). Most isolates of Shubra (89%) and Indiana (88%) were resistant to ≥9 antimicrobials, compared to only 11% of Enteritidis and 9% of Infantis that showed similar resistance. Class I integrons were detected in 10% of the isolates, and contained *aadA*, *tetR*, *dhfr*, bla_{PSE-1}, bla_{DHA-1} and bla_{VEB-1} gene cassettes alone or various combinations. Ceftriaxone- and/or cefoperazone-resistant isolates ($n=62$) carried bla_{TEM} (51.6%) and/or bla_{CMY-2} (56.5%). A total of 116 PFGE patterns were generated among 210 selected isolates. Our findings indicated that *Salmonella* contamination was common in retail meats, and that the *Salmonella* isolates were phenotypically and genetically diverse. Additionally, many *Salmonella* isolates were resistant to multiple antimicrobials.

1. Introduction

Foodborne diseases caused by nontyphoid *Salmonella* represent an important public health problem worldwide. It is estimated that approximately 70%–80% of foodborne bacterial outbreaks were caused by *Salmonella* in China (Wang et al., 2007). In the United States, 164,044 *Salmonella* infections (approximately 32,000 annually) were reported during 1998–2002 (Lynch et al., 2006). Pork, beef and poultry/chicken meat have been recognized as significant sources of human salmonellosis (Magistrali et al., 2008). Currently, ceftriaxone is the drug of choice for treating salmonellosis, especially in children because of its pharmacodynamic properties and the very low prevalence of resistance to the agent (Fey et al., 2000).

Although more than 2500 serovars of *Salmonella enterica* have been identified, most human *Salmonella* infections are caused by a limited number of serovars. *S. enterica* Typhimurium and Enteritidis are the most common causes of human salmonellosis worldwide, whereas serovars including Derby and Indiana have been reported to be prevalent in certain foods and regions (Bangtrakulnonth et al., 2004; Herikstad et al., 2002; Galanis et al., 2006; Xia et al., 2009). Serotyping and pulse field gel electrophoresis (PFGE) are effective surveillance tools to detect outbreaks, identify outbreak source, monitor trends over time, and attribute human disease to various foods and animals (Galanis et al., 2006).

In the past 20 years, the emergence and spread of antimicrobial-resistant *Salmonella*, particularly multidrug resistant (MDR) strains, is one of major public health concerns, and most infections with MDR *Salmonella* are acquired by eating contaminated foods of animal origin (White et al., 2001). Recently, an alarming increase in MDR *Salmonella* and the occurrence of emerging *Salmonella* serovars have been reported in many European and Asian countries (Cailhol et al., 2006; Cui et al.,

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2008; Hoge et al., 1998; Jones et al., 2002; Lauderdale et al., 2006; Pan et al., 2009; Van et al., 2003; Xia et al., 2009). However, there is a paucity on data regarding the prevalence, types of serovars, PFGE profiles and antimicrobial susceptibility of *Salmonella* from retail meats in China.

In this study, we characterized *Salmonella* isolates recovered from retail meats in 2007 and 2008 in Shaanxi Province, China. Our findings indicated that *Salmonella* contamination in retail meats was common, and that the *Salmonella* isolates were phenotypically and genetically diverse. Many *Salmonella* isolates were resistant to multiple antimicrobials.

2. Materials and methods

2.1. Isolation and identification of Salmonella

A total of 764 retail meat samples including 515 chicken, 91 pork, 78 beef and 80 lambs were collected monthly during 2007–2008 in supermarkets and free markets in Xian, Yangling and Baoji, which are located in the east, centre, and west, respectively, in Shaanxi Province, China. The meat samples were tested for *Salmonella* using previously described methods (Cui et al, 2006). Briefly, 25 g of ground meat was placed into a sterile glass flask containing 225 ml of buffered peptone water (BPW, Difco, Cockeysville, MD). For chicken carcasses, a whole piece was placed in a plastic bag and washed with 400 ml BPW with shaking vigorously for 2 min. The rinse was incubated at 37 °C in a water bath with shaking at 100 rpm for 6 h. After the pre-enrichment, 10 ml and 1 ml cultures were transferred to 100 ml each of the tetrathionate (TT, Difco) and Rappaport–Vassiliadis (RV, Difco) broth, respectively. The TT and RV broth were incubated at 42 °C in a water bath with shaking at 100 rpm for 24 h, followed by streaking TT onto xylose lysine tergitol 4 (XLT4, Difco) agar and RV onto xylose lysine desoxycholate (XLD, Difco) agar. After incubation for 48 h at 35 °C, two of presumptive *Salmonella* colonies were picked from each plate and stabbed into triple sugar iron (TSI, Difco) and urea-agar (Difco) slants, respectively, incubated for 24 h at 35 °C. Isolates with typical *Salmonella* phenotypes were confirmed by PCR using primers *inv*AF (5′-GTGAAATTATCGCCACGTTCGGGCAA-3′) and *inv*AR (5′-TCATCGCACCGTCAAAGGAACC-3′). The PCR reaction was carried out in a 25 μL PCR mixture containing 0.5 μM of each primer, 250 μM of dNTP, 1×PCR buffer, 1.5 mM $MgCl_2$, 0.5 U of Taq DNA polymerase (TaKaRa, Dalian, China) and 5 μL of sample DNA, using a mycircle PCR system (Bio-Rad, Hercules, CA) with incubation at 94 °C for 10 min, followed by 35 cycles of 94 °C for 30 s, 64 °C for 30 s and 72 °C for 30 s, and a final extension of 72 °C for 7 min. PCR products were stained with ethidium bromide and visualized under UV light after gel electrophoresis on 1% agarose.

2.2. Serotyping

All isolates were serotyped in Henan Center for Disease Control and Prevention, Zhengzhou, Henan, China. O and H antigens were characterized using slide agglutination with hyperimmune sera (S&A Company, Thailand) and the serotype was assigned following the manufacturer's instructions.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility profiles were determined via agar dilution according to the standards and guidelines described by the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2003). The following antimicrobials were tested: amikacin, amoxicillin/clavulanic acid, ampicillin, ceftriaxone, cefoperazone, cefoxitin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline and trimethoprim/sulfamethoxazole. *Escherichia coli* ATCC 25922 and ATCC 35218, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were used as quality control organisms in antimicrobial minimum inhibitory concentrations (MICs) determinations.

2.4. Detection of class I integrons and resistance genes

Sulfamethoxazole-resistant *Salmonella* isolates were further screened for the presence of class I integrons. In addition, isolates that demonstrated resistance to the extended-spectrum ceftriaxone and cefoperazone were examined for the presence of the extended-spectrum β-lactamase genes bla_{CMY-2} and bla_{TEM}. Class I integrons were amplified with primers 5′-CS (5′-GGCATCCAAGCAGCAAGC-3′) and 3′-CS (5′-AAGCAGACTTGACCTGAT-3′) using previously described methods (Yang, et al., 2009). Bla_{CMY-2} and bla_{TEM} were amplified as previously described (Chen, et al., 2004; Hasman et al., 2005).

2.5. PFGE

PFGE was performed according to the protocol developed by the Centers for Disease Control and Prevention (CDC) (Ribot et al., 2006). Briefly, agarose-embedded DNA was digested with 50 U of *Xba*I (TaKaRa) for 1.5–2 h in a water bath at 37 °C. The restriction fragments were separated by electrophoresis in 0.5×TBE buffer at 14 °C for 18 h using a Chef Mapper electrophoresis system (Bio-Rad) with pulse times of 2.16–63.8 s. *S.* Braenderup H9812 was used as the control strain. The gels were stained with ethidium bromide, and DNA bands were visualized with UV trans-illumination (Bio-Rad). PFGE results were analyzed using the BioNumerics Software (Applied-Maths, Kortrijk, Belgium).

2.6. Nucleotide sequencing analysis

PCR products of integrons were purified with a kit (TaKaRa). The DNA sequences of the PCR products were determined at Beijing AuGCT biotechnology Co., Ltd, and aligned using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/).

3. Results

3.1. Isolation and identification of Salmonella

Of the 764 retail meat samples, 333 (44%) were positive of *Salmonella*, including 276 (54%) of the 515 chicken, 28 (31%) of the 91 pork, 13 (17%) of the 78 beef and 16 (20%) of the 80 lamb samples (Table 1). A total of 359 *Salmonella* isolates were collected from the 333 *Salmonella*-positive samples (1–3 isolates per sample) including 292 isolates from chicken, 38 from pork, 13 from beef and 16 from lamb.

Twenty-four serovars were identified among the 359 *Salmonella* isolates. The top 10 serovars were Enteritidis ($n=113$), Typhimurium ($n=48$), Shubra ($n=36$), Indiana ($n=35$), Derby ($n=34$), Djugu ($n=25$), Infantis ($n=11$), Agona ($n=9$), Othmarschen ($n=8$) and Virchow ($n=8$) (Table 2). The most commonly recovered serovars from different retail meats were Enteritidis, Typhimurium, Shubra and Indiana from chicken; Derby, Enteritidis and Typhimurium from pork; Typhimurium, Derby and Agona from beef; and Derby, Djugu and Saintpaul from lamb. Several unusual serovars including Pakistan, Rideau and Bsilla were recovered from pork and beef, whereas Othmarschen, Tennessee, Thompson, Galiema, Kallo, Salmonella IIIa,

Table 1
Prevalence of *Salmonella* in retail meats.

Meat	No. samples	No. (%) samples positive for *Salmonella*	No. *Salmonella* isolates recovered[a]
Chicken	515	276 (54)	292
Pork	91	28 (31)	38
Beef	78	13 (17)	13
Lamb	80	16 (20)	16
Total	764	333 (44)	359

[a] 1–3 *Salmonella* isolates were collected from each positive sample.

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

Serovar	No. isolate	Type of meat				Serovar	No. isolate	Type of meat			
		Chicken	Pork	Beef	Lamb			Chicken	Pork	Beef	Lamb
Enteritidis	113	104	7	1	1	Rideau	4	0	4	0	0
Typhimurium	48	38	6	2	2	Tennessee	3	3	0	0	0
Shubra	36	34	0	1	1	Thompson	2	2	0	0	0
Indiana	35	32	1	1	1	Galiema	2	2	0	0	0
Derby	34	15	14	2	3	Kallo	2	2	0	0	0
Djugu	25	21	0	1	3	IIIa	2	2	0	0	0
Infantis	11	9	0	1	1	Rissen	1	1	0	0	0
Agona	9	2	4	1	2	Brancaster	1	1	0	0	0
Othmarschen	8	8	0	0	0	Braenderup	1	1	0	0	0
Virchow	8	7	0	1	0	Litchfield	1	1	0	0	0
Salmonella II	7	6	1	0	0	Pakistan	1	0	1	0	0
Saintpaul	4	1	0	1	2	Bsilla	1	0	0	1	0

Rissen, Brancaster, Braenderup and Litchfield were isolated from chicken. In contrast, other serovars such as Enteritidis, Typhimurium and Indiana were recovered from each of the four types of retail meats (Table 2).

3.2. Antimicrobial susceptibility

Two hundred and eighty-four (79%) *Salmonella* isolates were resistant to at least one antimicrobial, and 252 (70%) to three or more, 55 (15%) to 13 or more. Resistance was most frequently observed to sulfamethoxazole (67%), followed by trimethoprim/sulfamethoxazole (58%) and tetracycline (56%), and to a lesser extent kanamycin (37%), nalidixic acid (35%), ampicillin (33%), amoxicillin–clavulanic acid (32%) and ceftriaxone (16%) (Table 3). Resistance to beta-lactam antimicrobials was most common among the chicken isolates, 11% of which exhibited cefoxitin resistance compared to 5% and 0% of pork and beef isolates, respectively (Table 3). Ceftriaxone resistance (19%) was only observed among the chicken isolates, and ciprofloxacin resistance (26%) was also primarily seen in chicken isolates. Similar resistance to tetracycline was found among chicken (57%), pork (53%), beef (46%) and lamb (56%) isolates.

When analyzed by types of retail meats, chicken isolates showed the highest rate of resistance to at least one antimicrobial (80%), followed by those recovered from pork (79%), lamb (69%) and beef (62%) (Table 4). The percentages of resistance to 4–6 antimicrobials were similar (11–15%) among *Salmonella* from the four meat products. However, approximately 28% of chicken, 24% of pork isolates were resistant to ≥9 antimicrobials compared to 8% of beef and 7% of lamb isolates (Table 4).

When analyzed by serovar, *Salmonella* isolates most frequently exhibiting resistance to 1–3 antimicrobials were *Salmonella* II (57%), Derby (50%), Enteritidis (44%), Typhimurium (25%) and Agona (22%) (Table 5). Isolates displaying resistance to 4–6 antimicrobials were Rideau (100%), Virchow (63%), Saintpaul (50%), Agona (44%) and Infantis (36%). Those isolates commonly showing resistance to ≥9 antimicrobials were observed among several serovars including Shubra (89%), Indiana (88%) and Typhimurium (27%). Most *Salmonella* serovars displayed resistance to examined antimicrobials except Othmarschen and Djugu (Table 5).

3.3. Presence of class I integrons and beta-lactamase genes

Class I integrons were identified in 37 *Salmonella* isolates (10%). Different integron profiles including 0.75 kb, 1 kb, 1.2 kb, 1.4 kb, 1.8 kb and 2 kb integrons were identified, with the most common being a 1.4 kb integron ($n=14$), followed by a 1.2 kb integron ($n=7$). One isolate yielded two different integrons. Ten *Salmonella* isolates, representing different retail meats, serovars and integrons, were selected for DNA sequence analysis (Table 6). Five resistance gene cassettes were identified, including *aadA*, *tetR*, *dhfr*, bla_{PSE-1}, bla_{DHA-1} and bla_{VEB-1}, which encode resistance to streptomycin, tetracycline,

Table 3
Antimicrobial resistance phenotypes of *Salmonella* isolated from retail meats.

Antimicrobial agent	% Resistance					
	Resistant breakpoint[a] (μg/ml)	Chicken ($n=292$)	Pork ($n=38$)	Beef ($n=13$)	Lamb ($n=16$)	Total ($n=359$)
Ampicillin	≥32	36	18	15	25	33
Amoxicillin/clavulanic acid	≥32	36	26	0	6	32
Cefoxitin	≥32	11	5	0	0	9
Cefoperazone	≥64	10	3	0	0	8
Ceftriaxone	≥64	19	0	0	0	16
Chloramphenicol	≥32	29	26	0	6	26
Tetracycline	≥16	57	53	46	56	56
Amikacin	≥32	19	0	8	6	16
Kanamycin	≥64	41	26	0	12	37
Gentamicin	≥16	30	8	0	6	26
Streptomycin[b]	≥64	31	32	0	6	29
Sulfamethoxazole	≥350	66	61	92	88	67
Trimethoprim/sulfamethoxazole	≥8/152	56	47	77	69	58
Nalidixic acid	≥32	39	29	8	6	35
Ciprofloxacin	≥4	26	3	0	0	21

[a] MICs (μg/ml) determined via agar dilution in accordance with CLSI.
[b] No CLSI breakpoint.

Table 4
Multidrug resistance (MDR) observed among *Salmonella* isolates recovered from retail meats.

Retail meats (no. isolates)	% Resistant to indicated number of antimicrobials				
	1–3	4–6	7–9	≥9	Total resistance (≥1)
Chicken ($n=292$)	25	11	16	28	80
Pork ($n=38$)	39	11	5	24	79
Beef ($n=13$)	23	15	15	8	62
Lamb ($n=16$)	25	13	25	7	69
Total ($n=359$)	26	11	15	26	79

trimethoprim and beta-lactamase, respectively. One Enteritidis isolate from chicken contained two integrons (1.2/1.8) carrying three resistance genes (bla_{PSE-1}/*dhfr*17-*aad*A5). Four 1.4 kb integrons carry four different resistance genes (Table 5).

Ceftriaxone and/or cefoperazone resistance were observed among 62 isolates (17.2%) representing 8 different serovars (Kallo, Derby, Enteritidis, Tennessee, Djugu, Shubra, Typhimurium and Indiana) recovered primarily from chicken. bla_{TEM} was identified in 32 ceftriaxone and/or cefoperazone resistant isolates (51.6%), and bla_{CMY-2} in 35 resistant isolates (56.5%).

3.4. PFGE

A total of 210 *Salmonella* isolates including serovars Enteritidis, Typhimurium, Shubra and Indiana were analyzed for genetic relatedness using PFGE with *XbaI*.

Fifty-two different PFGE patterns were observed among the 109 isolates of Enteritidis (Fig. 1). Using a cutoff value of 96% similarity, six clusters and several individual types were observed. The most common clusters consisted of 57 and 38 isolates, respectively (data not shown). In the first cluster, although these isolates were mainly recovered from chicken ($n=51$), the samples were collected from different districts (44 from Yangling, 11 from Xian and 2 from Baoji) in different times over a year (data not shown). PFGE analysis also exhibited good correlations with antimicrobial resistance phenotypes (data not shown).

The Typhimurium isolates ($n=35$) were assigned to 19 different PFGE patterns (Fig. 2). Eleven (31.4%) isolates shared one PFGE pattern. Using a cutoff value of 95% similarity, six clusters and a few individual types were observed. The most common clusters consisted of 8 and 15 isolates, respectively. Twenty-eight (80%) isolates in this serovar recovered from chicken, 4 from pork, 2 from lamb and 1 from beef.

The Shubra isolates ($n=35$) were assigned to 23 different PFGE patterns (Fig. 3). Using a cutoff value of 95% similarity, six clusters and some individual types were observed. The most common cluster consisted of 13 isolates. Thirty-three isolates (94%) in this serovar recovered from chicken except 1 from pork and 1 from lamb.

Indiana isolates ($n=31$) were assigned to 22 different patterns (Fig. 4). Unlike the other serovars, no predominant PFGE type was found.

Table 5
Multidrug resistance (MDR) observed among *Salmonella* serovars obtained from retail meats.

Serovars (no. isolates)	% Resistant to indicated number of antimicrobials				
	1–3	4–6	7–9	≥9	Total resistance (≥1)
Enteritidis ($n=113$)	44	11	12	11	77
Typhimurium ($n=48$)	25	19	13	27	83
Shubra ($n=36$)	0	0	11	89	100
Indiana ($n=35$)	0	3	9	88	100
Derby ($n=34$)	50	15	18	3	94
Djugu ($n=25$)	8	36	4	0	48
Infantis ($n=11$)	18	36	9	9	73
Agona ($n=9$)	22	44	11	0	78
Othmarschen ($n=8$)	0	0	13	0	13
Virchow ($n=8$)	0	63	25	0	88
Salmonella II ($n=7$)	57	14	29	0	100
Saintpaul ($n=4$)	0	50	50	0	100
Rideau ($n=4$)	0	100	0	0	100
Total ($n=359$)	25	16	12	25	79

Table 6
Representative class I integron-associated resistance genes among *Salmonella* isolates recovered from retail meats ($n=10$).

Isolate #	Serovar	Source	Integron (kb)	Gene cassette[a]
1–24	Agomal	Beef	0.75	*aad*A2
1–13	Virchow	Pork	1	*tet*R
S26	Enteritidis	Chicken	1.2	bla_{PSE-1}
1–11	Virchow	Beef	1.4	*aad*A1–*dhfr*I
2–43	Indiana	Pork	1.4	*aad*A2–bla_{DHA-1}
2–31	Galiema	Chicken	1.4	*dhfr*V–unknown
2–30	Galiema	Chicken	1.4	*aad*A1–bla_{VEB-1}
J55	Shubra	Chicken	1.8	*dhfr*XII–*aad*A2
J91	Shubra	Chicken	1.8	*dhfr*XII–*aad*A2
S8	Enteritidis	Chicken	1.2/1.8	bla_{PSE-1}/*dhfr*17–*aad*A5

[a] aadA1/aadA2/aadA5 encode resistance to streptomycin/spectinomycin, *tet*R encode resistance to tetracycline, bla_{PSE-1}/bla_{DHA-1}/bla_{VEB-1} encode resistance to beta-lactam antibiotics, *dhfr*1/*dhfr*V/*dhfr*VII/*dhfr*17 encode resistance to trimethoprim, unknown is gene cassette of unknown function.

4. Discussion

In this study, we screened retail meat products collected from four districts of Shaanxi Province in China for the presence of *Salmonella*, and further characterized *Salmonella* isolates using serotyping, antimicrobial susceptibility and PFGE.

The present study 44% of the retail meats and 54% of the chicken samples were contaminated with *Salmonella*, compared to 38% reported in Henan Province, a neighbor province of Shaanxi (Zhang et al, 2009), 60% in Spain (Carraminana et al., 1997), 61–69% in Canada (Lammerding et al., 1988), 69% in Greece (Arvanitidou et al., 1998), and 6–35% in the United States (White et al., 2001). However, several factors must be taken into account when making such comparisons, including differences in country and origin, type of meat samples, sampling seasons, slaughterhouse sanitation, and isolation methods.

Enteritidis was the most common serovar identified, particularly in chicken meats. This serovar was also found to be a predominant serovar in poultry products in other survey studies (Arvanitidou et al., 1998; Machado and Bernardo, 1990; Plummer et al., 1995; Uyttendaele et al., 1998), as well as one of the most common serovars that cause human salmonellosis in many countries. Although, to our knowledge, no reports on the occurrence of *Salmonella* serovars isolated from humans in Shaanxi Province, Xia (2009) reported that in Henan Province, the second most common *Salmonella* serovar isolated from humans was Enteritidis (17%). Data from Asia, Europe and Latin America have indicated that Enteritidis is the most common serovar among human isolates (Galanis et al., 2006) Other serovars that were repeatedly recovered in the present study included Typhimurium, Shubra, Indiana and Derby. The findings were in agreement with those described in previous studies showing that Typhimurium, Enteritidis and Derby are the most common serovars worldwide, poultry meats and eggs are the major reservoir of Enteritidis, whereas Typhimurium is widespread (Bangtrakulnonth et al., 2004; Cui et al., 2005; Guard-Petter, 2001; Herikstad et al., 2002; Olsen et al., 2001; Xia et al., 2009). Other serovars such as Newport, Heidelberg, Weltevreden and Kentucky, that are

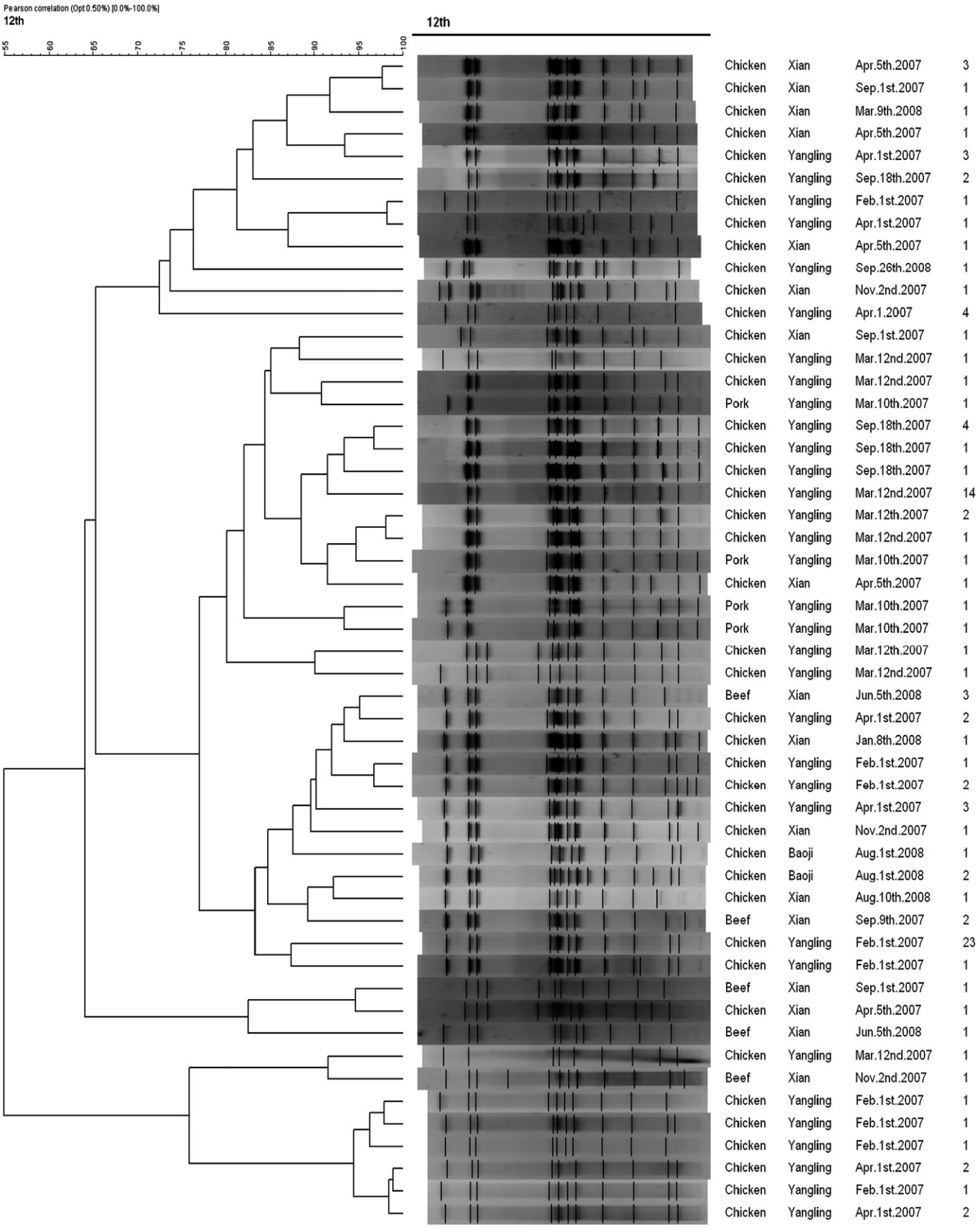

Fig. 1. Dendrogram of 52 PFGE patterns of 109 *Salmonella* Enteritidis isolates from retail meats in Shaanxi Province, China.

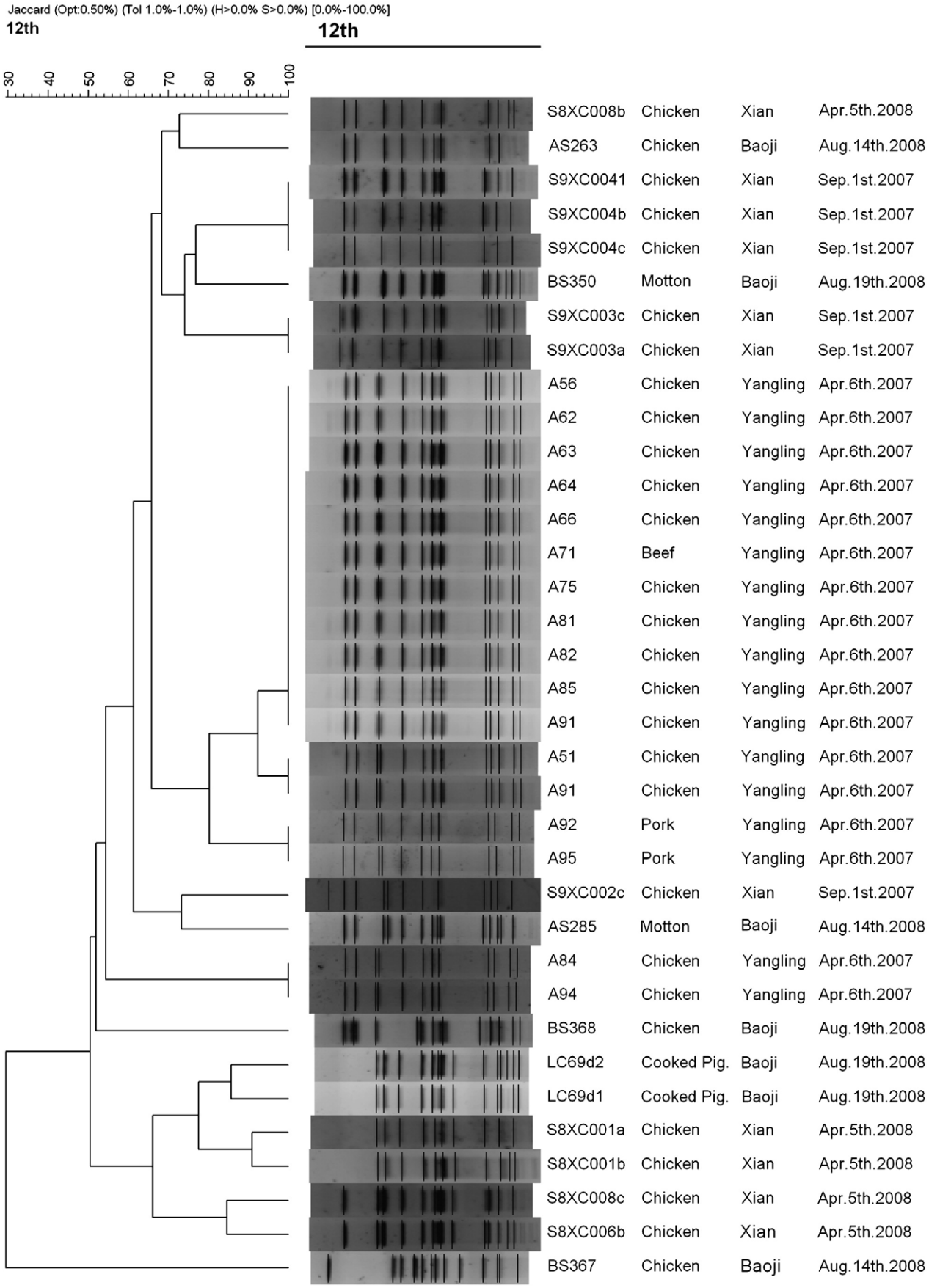

Fig. 2. Dendrogram of PFGE patterns of 35 *Salmonella* Typhimurium isolates from retail meats in Shaanxi Province, China.

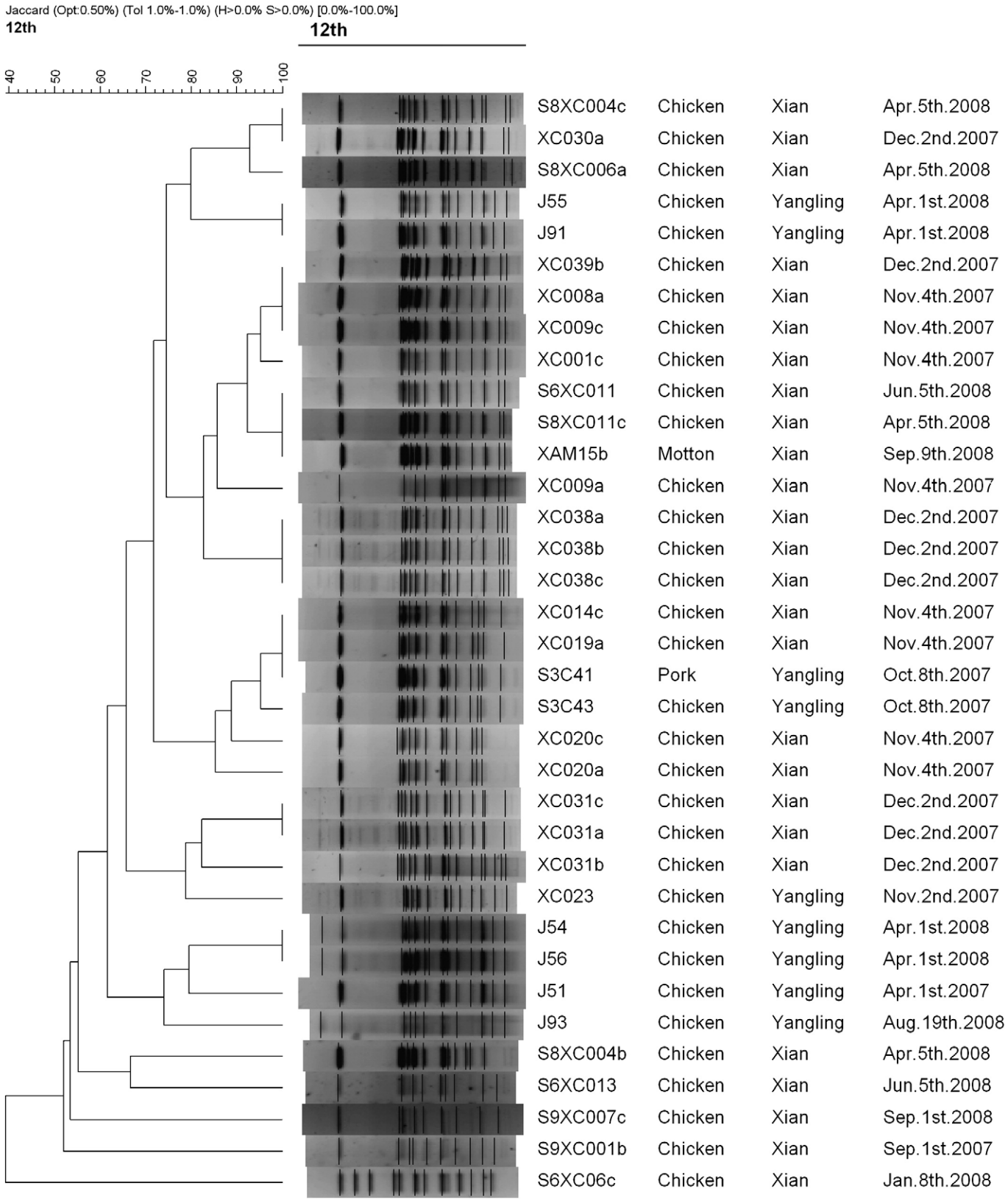

Fig. 3. Dendrogram of PFGE patterns of 35 *Salmonella* Shubra isolates from retail meats in Shaanxi Province, China.

common in many countries, were rarely detected in Shaanxi Province (Chen et al., 2004; Galanis et al., 2006; Herikstad et al., 2002).

Antimicrobial resistance in *Salmonella* has become a significant public health concern worldwide. Surveillance data demonstrated a noticeable increase in overall antimicrobial resistance among salmonellae from 20%–30% in the early 1990s to as high as 70% in some countries in 2000s (Su et al., 2004). Our results indicated that only 75 of 359 (21%) of the *Salmonella* isolates were susceptible to the antimicrobial agents tested, whereas nearly 80% of the isolates were resistant to at least one antimicrobial agent, 26% to more than 9

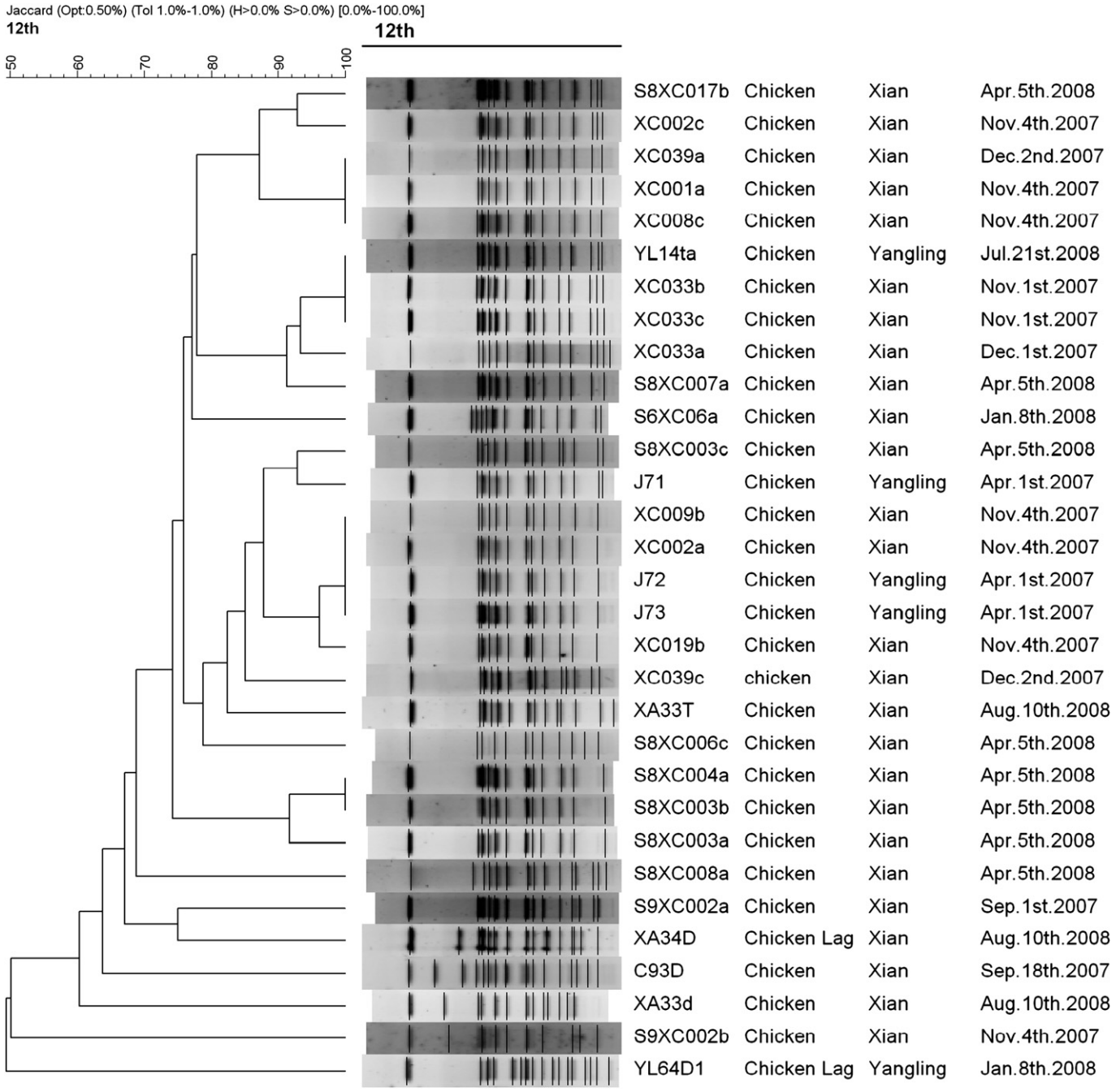

Fig. 4. Dendrogram of PFGE patterns of 31 *Salmonella* Indiana isolates from retail meats in Shaanxi Province, China.

antimicrobials. A high percentage of antimicrobial resistant *Salmonella* from retail meats was also reported by several investigators (Arvanitidou et al., 1998; Bokanyi et al., 1990; Chen et al., 2004; D'Aoust et al., 1992). As animals are a main reservoir of *Salmonella* and the use of antimicrobial 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* (Barbosa and Levy, 2000). Of the serovars identified in the present study, Shubra, Indiana, Virchow and Derby showed the highest rate of antimicrobial resistance and multidrug resistance, whereas Enteritidis was relatively more susceptible to antimicrobial agents, which was in agreement with previous observations among *Salmonella* recovered from retail meats and food animals (Su et al. 2004; Zhao et al., 2007), but differed to some reports (Antunes et al., 2003; Chen et al., 2004). Among human clinical isolates recovered in China, a recent study reported that 31 ciprofloxacin-resistant clinical *Salmonella* isolates were also resistant to ≥8 other antimicrobial drugs (Cui et al., 2008). In Henan Province, 54% of human isolates were MDR strains (Xia et al., 2009).

In the present study, resistance to nalidixic acid and ciprofloxacin was very common (35% and 21%, respectively), particularly among isolates recovered from chicken and pork meats. Other reports from China and several countries also indicated an increase in ciprofloxacin-resistant *Salmonella* (Cailhol et al., 2006; Cui et al., 2008; Lauderdale et al., 2006). As infections with ciprofloxacin-resistant *Salmonellae* are associated with increased morbidity and mortality, and ciprofloxacin-resistant *Salmonella* are usually resistant to multiple drugs (Cui et al., 2008), it presents an enormous challenge to the treatment of *Salmonella* infections in humans and animals.

Ceftriaxone is another commonly used drug to treat children with *Salmonella* infections, particularly in invasive infections, due to its favorable pharmacokinetic properties and low prevalence of resistance (White et al., 2001). The increased prevalence of ceftriaxone-resistant *Salmonella* strains in food animals may be related to the veterinary use of ceftiofur, an expanded-spectrum cephalosporin used only in veterinary medicine (Carattoli et al., 2002). Resistance to ceftriaxone is largely due to the AmpC β-lactamase (bla_{CMY-2} and bla_{TEM}) genes (Chen et al., 2004; White et al., 2001). In this study, more than 50% ceftriaxone and/or cefoperazone-resistant isolates harbored bla_{TEM} or bla_{CMY-2}. The dissemination of *Salmonella* resistant to multiple drugs, including cephalosporins, through food has important public health implications.

Integrons, a mobile DNA element, are well known for their ability to transfer resistance and often contain one or more linked antimicrobial-resistance genes (Hall, 1997; Hall and Stokes, 1993). Our data showed that class I integrons were widely spread in different *Salmonella* serovars recovered from different retail meats. Several serovars including Agona, Virchow, Enteritidis, Indiana, Galiema and Shubra contained integrons in size from 0.75 kb to 1.8 kb, which harbored *aad*A2, bla_{PSE-1}, *dhfr*XII-*aad*A2 and *tet*R, confirming that integrons play an important part in the transfer of resistance among bacteria (Cloeckaert et al., 2000; Guerra et al., 2000; White et al., 2001; Zhao et al., 2007).

The PFGE results indicated a genetically diverse *Salmonella* population, whereas several indistinguishable PFGE patterns were shared among isolates within certain serovars obtained from different retail meats. For example, two clusters of 23 and 14 of Enteritidis isolates that recovered from different retail meats, different time and different districts showed the same PFGE patterns. The majority of these isolates exhibited similar resistance profile. Similar results were also seen in Typhimurium and Indiana.

In conclusion, our findings demonstrated that *Salmonella* contamination was common in retail meats, and that the *Salmonella* isolates were diverse both phenotypically and genetically. Additionally, many *Salmonella* isolates were resistant to multiple antimicrobials, and multidrug resistance was strongly associated with types of serovars. Surveillance programs concerning the prevalence of foodborne pathogens and their antimicrobial resistance and subtyping profiles are important for conducting epidemiological investigations and for monitoring the development and dissemination of antimicrobial resistance. Such information is needed for making science based public health policy, and developing effective intervention strategies including implementation of hazard analysis of critical control point programs in food production to ensure the safety of our food supplies.

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