

Prevalence and Fimbrial Genotype Distribution of Poultry *Salmonella* Isolates in China (2006 to 2012)

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In this study, a total of 323 *Salmonella enterica* strains were isolated from 3,566 rectal swab samples of 51 poultry farms in seven regions of 12 provinces of China between 2006 and 2012. The prevalences of *Salmonella* sp. carriage were 12.4% in geese (66 positive/533 samples), 10.4% in turkeys (32/309), 9.8% in chickens (167/1,706), 6.8% in ducks (41/601), and 4.1% in pigeons (17/417), respectively. These isolates belonged to 20 serovars, in which the most frequent serovars were *S. enterica* serovar Gallinarum biovar Pullorum (herein, *S. Pullorum*) (55 isolates, 17.0%), *S. enterica* serovar Typhimurium (50 isolates, 15.5%), and *S. enterica* serovar Enteritidis (39 isolates, 12.1%). Overall, *S. Typhimurium* was the most commonly detected serovar; among the individual species, *S. Pullorum* was most commonly isolated from chickens, *S. Enteritidis* was most common in ducks, *S. Typhimurium* was most common in geese and pigeons, and *S. enterica* serovar Saintpaul was most common in turkeys. PCR determination of 20 fimbrial genes demonstrated the presence of *bcfD*, *csgA*, *fimA*, *stdB*, and *sthE* genes and the absence of *staA* and *stgA* genes in these isolates, and other loci were variably distributed, with frequency values ranging from 11.8 to 99.1%. These 323 *Salmonella* isolates were subdivided into 41 different fimbrial genotypes, and of these isolate, 285 strains (88.2%) had 12 to 14 fimbrial genes. Our findings indicated that the *Salmonella* isolates from different poultry species were phenotypically and genetically diverse and that some fimbrial genes are more frequently associated with serovars or serogroups.

Salmonella spp. are important zoonotic pathogens which cause significant morbidity, mortality, and economic losses (1, 2). It has been estimated that there are 1.3 billion cases of human gastroenteritis due to *Salmonella* each year worldwide, and these result in 3 million deaths (3). Poultry is considered a major reservoir for many serovars of *Salmonella*, and often human infection is attributed to consumption of contaminated poultry products such as eggs and meats (4).

Currently, the genus *Salmonella* consists of only two species, *Salmonella bongori* and *Salmonella enterica*, with the latter containing *Salmonella* subspecies: *S. enterica* subsp. *enterica* or I, *S. enterica* subsp. *salamae* or II, *S. enterica* subsp. *arizonae* or IIIa, *S. enterica* subsp. *diarizonae* or IIIb, *S. enterica* subsp. *houtenae* or IV, and *S. enterica* subsp. *indica* or VI (5, 6). Using the White-Kauffmann-Le Minor Scheme based on somatic, flagellar, and capsular antigens, over 2,600 serovars have been identified (5, 6). The prevalence of *Salmonella* serovars in poultry varies in different countries and also over time (7, 8). Certain serovars emerge within a country or region for a period and then disappear with no obvious cause or intervention measure.

In addition to the somatic, flagellar, and capsular antigens, other surface-exposed components of *Salmonella* have been the targets of evolutionary adaptation to changing selective conditions of the environment. The ability to adhere to the host's epithelial cells is considered a prerequisite for successful infection, and fimbriae, the proteinaceous hair-like appendages on the outer membrane of bacteria, have been implicated in such adherence (9). Previous studies have shown that some fimbrial proteins are carried by all *Salmonella* serovars while others are restricted to certain serovars with particular hosts, suggesting a potential role for fimbriae in regulating host specificity (10, 11). *Salmonella* carries different types of fimbriae, with each serovar having its struc-

tural subunit and biogenesis genes carried by one fimbrial gene cluster (FGC). Genotyping of the FGCs of *Salmonella* might, then, facilitate the determination of phylogenetic relationships between strains and also provide information on their host specificity, pathogenicity, and transmission efficiencies (12).

The aims of this study were to (i) evaluate the prevalence of *Salmonella* in various poultry species on different farms in China, (ii) investigate the diversity of *Salmonella* serovars, and (iii) illustrate the distribution of fimbrial genotypes in *Salmonella* isolates.

MATERIALS AND METHODS

All work in this study was reviewed and approved by the Institutional Animal Care and Use Committee of the Poultry Institute, Chinese Academy of Agricultural Sciences.

Sample collection. Rectal swabs were made randomly from individual healthy birds in different houses or coops in each farm as described previously (13). Each swab was placed in a sterile plastic bag and transported at ambient temperature to the laboratory, where it was stored at 4°C until examined.

Isolation and identification of *Salmonella*. Swabs were cultured in 9 ml of Gram-negative (GN) broth (Tianhe, Hangzhou, China) at 37°C for

Received 26 September 2013 Accepted 6 November 2013

Published ahead of print 15 November 2013

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Supplemental material for this article may be found at <http://dx.doi.org/10.1128/AEM.03223-13>.

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doi:10.1128/AEM.03223-13

TABLE 1 *Salmonella* spp. isolated from poultry in 12 provinces of China

Province	No. of positive samples/no. of samples tested (%) ^a					
	Chicken	Duck	Goose	Pigeon	Turkey	Total
Jiangsu	73/685 (11)	10/157 (6)	22/205 (11)	6/122 (5)	8/93 (9)	119/1,262 (9)
Anhui	9/74 (12)	7/66 (11)	13/95 (14)	4/50 (8)	8/64 (13)	41/349 (12)
Zhejiang	14/169 (8)	11/189 (6)	8/67 (12)			33/425 (8)
Shandong	9/161 (6)	5/67 (8)	11/42 (26)		9/52 (17)	34/322 (11)
Shanghai	3/58 (5)			1/54 (2)		4/112 (4)
Hebei	9/123 (7)			3/55 (6)		12/178 (7)
Beijing	4/55 (7)					4/55 (7)
Sichuan		5/73 (7)	5/42 (12)			10/115 (9)
Henan	8/64 (13)	3/49 (6)		2/73 (3)	5/47 (11)	18/233 (8)
Xinjiang	36/278 (13)					36/278 (13)
Gungdong			3/35 (9)	1/63 (2)	2/53 (4)	6/151 (4)
Heilongjiang	2/39 (5)		4/47 (9)			6/86 (7)
Total	167/1,706 (10)	41/601 (7)	66/533 (12)	17/417 (4)	32/309 (10)	323/3,566 (9)

^a Not all types of samples were available for all provinces.

24 h before aliquots of 100 µl of the broth were streaked onto xylose lysine deoxycholate (Tianhe, Hangzhou, China) plates and incubated at 37°C for 24 h. Where typical *Salmonella* colonies were seen, they were further examined by the Voges-Proskauer (VP) and methyl red (MR) tests and by plating onto triple sugar iron (TSI), Christensen's urea, lysine iron agar (LIA), and mobility indole ornithine agar (Tianhe, Hangzhou, China). At least two colonies from each positive plate were maintained on brain heart infusion agar (Tianhe, Hangzhou, China) for subsequent PCR analysis.

Determination of serogroup and serovar. The serogroup of each *Salmonella* isolate was determined using a slide agglutination test with O-antigen antiserum while the serovar of each isolate was established with a tube agglutination test using H-antigen antiserum. The serovars of all strains identified as *S. enterica* were determined according to the Kauffmann-White serotyping scheme (6) with commercial antisera (S & A Reagent Laboratory LMT, Bangkok, Thailand). The identification of *S. enterica* serovar Gallinarum biovar Gallinarum (*S. Gallinarum*) and *S. Gallinarum* biovar Pullorum (*S. Pullorum*) was based on duplex PCR analysis as described previously (14).

PCR primers for fimbrial genes. The primers used in this study to amplify the fimbrial genes are listed in Table S1 in the supplemental material. PCR amplification of *bcfD*, *csfA*, *fimA*, *lpfD*, *pefA*, *safC*, *sefA*, *stbD*, *stfH*, *sthE*, *stiH*, *stjA*, and *tcfA* was performed as described previously (10, 15–19). PCR primers to amplify the *pegD*, *staA*, *stcA*, *stdB*, *stxB*, *stgA*, and *stkA* genes were established and validated in this study, and the gene accession numbers for the fimbrial genes are shown in Table S1 in the supplemental material together with the locations of the genes. All primers used in the study were obtained from Sangon Biological Engineering Technology and Service Co. Ltd. (Shanghai, China). Gel electrophoresis following PCR showed robust amplification of the target genes in the positive controls (reference strains *S. enterica* serovar Typhimurium, ATCC 14028; *S. enterica* serovar Enteritidis, ATCC 13076; *S. enterica* serovar Typhi, Center for Medical Culture Collections [CMCC; China]50071, and *S. enterica* serovar Heidelberg, CMCC 50111) and the absence of amplification in the negative control (*Pasteurella multocida*, Center for Veterinary Culture Collections [CVCC; China] 44801). The specificity of the PCR systems was further confirmed by DNA sequencing and a BLAST search of PCR products in the GenBank.

DNA extraction and PCR. All *Salmonella* sp. isolates and reference strains were grown on LB agar plates at 37°C overnight. A single bacterial colony was selected and suspended in 100 µl of deionized water and boiled for 10 min before being chilled on ice for 5 min. Following centrifugation at 10,000 × g for 5 min, the supernatant was removed and used as the DNA template in PCR amplifications.

All PCRs were performed on 1 µl of DNA in a final volume of 25 µl

using a PCR premix (TaKaRa, Dalian, China) according to the manufacturer's instructions. The PCR cycling conditions consisted of an initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. After a final extension at 72°C for 10 min, the DNA amplification products were identified by electrophoresis with 1.2% agarose gels.

PCR products of an appropriate size were purified from the gels using a Quick Gel Extraction Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions and sequenced at the DNA Synthesis and Sequencing Facility at Sangon Biological Engineering Technology and Service Co., Ltd. (Shanghai, China).

Data analysis. Data on the farms, species of poultry tested, and culture and PCR results were entered into a spreadsheet of Microsoft Excel 2003 and transferred to the statistical software program SPSS (version 13.0) for Windows (SPSS Inc., Chicago, IL, USA). Data were compared using a chi-square test, and differences were regarded as significant at a *P* value of ≤0.05.

RESULTS

***Salmonella* prevalence.** Between November 2006 and October 2012, 3,566 rectal swabs were collected from poultry on 51 farms in seven regions in China: eastern (Anhui, Jiangsu, Shandong, Shanghai, and Zhejiang provinces), central (Henan), northeastern (Heilongjiang), northwestern (Xinjiang), northern (Beijing and Hebei), southwestern (Sichuan), and southern (Guangdong) (Table 1 and Fig. 1; see also Table S2 and Fig. S1 in the supplemental material). The overall prevalence of *Salmonella* spp. was 9.1%, and the prevalence was 12.4% in geese, 10.4% in turkeys, 9.8% in chickens, 6.8% in ducks, and 4.1% in pigeons. *Salmonella* prevalence in the different poultry species varied considerably, with geese having the largest range (7.4 to 26.2%) and ducks having the smallest (3.4 to 10.6%). There was also considerable variation in the prevalence in the different regions surveyed (3.6 to 12.9%) and in the different years of the study (7.0 to 17.5%) (Fig. 1; see also Fig. S1).

A total of 323 *Salmonella* isolates were obtained, and 20 serovars were identified, with the most prevalent being *S. Pullorum* (*n* = 55; 17%), *S. Typhimurium* (*n* = 50; 15.5%), and *S. Enteritidis* (*n* = 39; 12%) (Table 2). The only serovar isolated from all five poultry species was *S. Typhimurium*, while *S. enterica* serovar Indiana, *S. Heidelberg*, and *S. enterica* serovar Kentucky were each

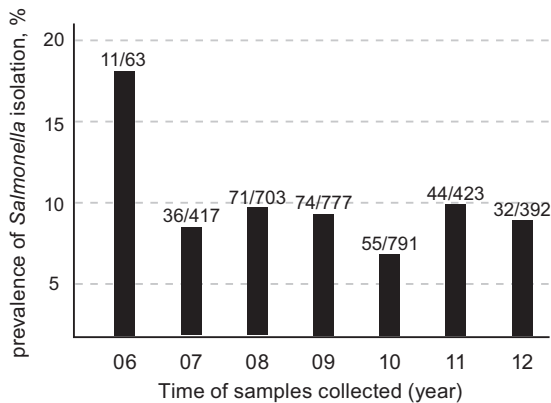


FIG 1 Prevalence of poultry *Salmonella* isolation between 2006 and 2012. The prevalence of poultry *Salmonella* spp. from 3,566 rectal swabs did not differ significantly between the sampling times of 2006 (17.5%, 11/63), 2007 (8.6%, 36/417), 2008 (10.1, 71/703), 2009 (9.5%, 74/777), 2010 (7.0%, 55/791), 2011 (10.4%, 44/423), and 2012 (9.1%, 323/3,566).

isolated from four species. *S. Gallinarum*, *S. enterica* serovar Bazenheid, *S. enterica* serovar Montevideo, *S. enterica* serovar Derby, *S. enterica* serovar Senftenberg, and *S. enterica* serovar Meleagridis were isolated only from a single species of bird (Table 2). The most common serovar isolated from chickens was *S. Pullorum*; *S. Enteritidis* was most common in ducks, *S. Typhimurium* was most common in geese and pigeons, and *S. enterica* serovar Saintpaul was most common in turkeys. *S. Pullorum* and *S. Gallinarum* were primarily isolated from chickens. The greatest diversity of serovars was found in chickens ($n = 18$), followed by geese (11), ducks (9), turkeys (8), and pigeons (3). Four serovars (*S. enterica* serovar Agona, *S. Meleagridis*, *S. Senftenberg*, and *S. enterica* serovar Anatum) existed in 1 of 12 provinces, while five serovars (*S.*

Pullorum, *S. Typhimurium*, *S. Enteritidis*, *S. Indiana*, and *S. enterica* serovar Postdam) were isolated from 6 or more provinces in this investigation (see Table S3 in the supplemental material).

Distribution of fimbrial genes. All 323 *Salmonella* isolates were further characterized by the molecular detection of 20 genes coding for bacterial fimbriae. All of the *Salmonella* isolates were positive by PCR for the *bcfD*, *csgA*, *fimA*, *stdB*, and *sthE* fimbrial genes and negative for the *staA* and *stgA* fimbrial genes (Table 3). The other fimbrial genes tested for were found in a variety of the isolates, with frequencies ranging from 11.8 to 99.1% (Table 3).

Based on the presence or absence of the 20 loci investigated in the study, the 323 *Salmonella* isolates could be subdivided into 41 different genotypes. On average, each serovar had two fimbrial genotypes while *S. Indiana*, *S. Typhimurium*, and *S. Heidelberg* isolated from multiple poultry species had over five fimbrial genotypes. Only a single fimbrial genotype was found for *S. Agona*, *S. enterica* serovar Reading, *S. enterica* serovar Thompson, *S. enterica* serovar Blockley, *S. enterica* serovar Bazenheid, *S. Meleagridis*, and *S. Senftenberg*. The *Salmonella* isolates we obtained had between 8 and 15 (average, 12.8) of the 20 fimbrial genes we studied, and 285 strains (88.2%) had 12 to 14 fimbrial genes. Two strains of *S. Montevideo* had the least number of fimbrial genes (8), whereas two strains of *S. Kentucky* and five strains of *S. Heidelberg* carried the most fimbrial genes (15).

Association of fimbrial genes with serovars/serogroups. Of the 20 fimbrial genes studied, the *bcfD*, *csgA*, *fimA*, *stdB*, *sthE*, *stbD*, *lpfD*, *stfH*, and *stiH* genes were present in all of the serovars while *staA* and *stgA* were always absent (Table 3 and Fig. 2). Of the remaining nine fimbrial genes, *safC* was absent only from serovar *S. Reading*; *steB* was absent from six serovars (*S. Typhimurium*, *S. Saintpaul*, *S. Reading*, *S. Montevideo*, *S. enterica* serovar Kottbus, and *S. Anatum*). *stjA* and *stcA* were absent from serogroup D1, *pegD* was mainly present in serogroup D1, *sefA* was only present in

TABLE 2 Poultry *Salmonella* serovars in this study

Serovar	No. of isolates from:					Total no. of isolates (%)
	Chicken	Duck	Goose	Pigeon	Turkey	
<i>S. Pullorum</i>	51	0	0	0	4	55 (17.0)
<i>S. Typhimurium</i>	22	2	11	10	5	50 (15.5)
<i>S. Enteritidis</i>	14	15	10	0	0	39 (12.1)
<i>S. Indiana</i>	9	3	10	0	3	25 (7.7)
<i>S. Heidelberg</i>	13	0	3	3	4	23 (7.1)
<i>S. Potsdam</i>	6	6	10	0	0	22 (6.8)
<i>S. Kentucky</i>	9	0	3	4	3	19 (5.9)
<i>S. Thompson</i>	8	0	6	0	2	16 (5.0)
<i>S. Saintpaul</i>	2	4	0	0	7	13 (4.0)
<i>S. Kottbus</i>	3	4	5	0	0	12 (3.7)
<i>S. Agona</i>	6	4	0	0	0	10 (3.1)
<i>S. Gallinarum</i>	9	0	0	0	0	9 (2.8)
<i>S. Blockley</i>	3	0	4	0	0	7 (2.2)
<i>S. Bazenheid</i>	5	0	0	0	0	5 (1.5)
<i>S. Anatum</i>	1	2	2	0	0	5 (1.5)
<i>S. Montevideo</i>	0	0	0	0	4	4 (1.2)
<i>S. Derby</i>	3	0	0	0	0	3 (0.9)
<i>S. Reading</i>	1	0	2	0	0	3 (0.9)
<i>S. Senftenberg</i>	2	0	0	0	0	2 (0.6)
<i>S. Meleagridis</i>	0	1	0	0	0	1 (0.3)
Total	167	41	66	17	32	323 (100)

TABLE 3 Numbers of the strains of the various *Salmonella* serovars isolated from poultry in China which contained the different fimbrial genes

Serogroup	Serovar	Strain no.	Fimbrial gene profile ^a												
			<i>lpfD</i>	<i>pefA</i>	<i>pegD</i>	<i>safC</i>	<i>sefA</i>	<i>stbD</i>	<i>stcA</i>	<i>steB</i>	<i>stfH</i>	<i>stiH</i>	<i>stjA</i>	<i>stkA</i>	<i>tcfA</i>
O:4(B)	<i>S. Typhimurium</i>	50	●	45	○	47	○	●	●	○	●	●	49	5	○
	<i>S. Indiana</i>	25	21	2	○	●	○	●	5	●	●	5	7	●	5
	<i>S. Heidelberg</i>	23	●	○	○	5	○	●	21	●	●	19	●	●	20
	<i>S. Saintpaul</i>	13	●	3	○	●	○	●	●	○	●	●	●	○	○
	<i>S. Agona</i>	10	●	○	○	●	○	●	●	●	●	●	●	○	○
	<i>S. Derby</i>	3	●	○	2	●	○	●	○	●	●	●	1	○	○
	<i>S. Reading</i>	3	●	○	○	○	○	●	○	○	○	○	●	●	○
O:7(C ₁)	<i>S. Potsdam</i>	22	●	3	○	●	○	19	●	●	●	●	●	○	○
	<i>S. Thompson</i>	16	●	○	○	●	○	●	○	●	●	●	○	○	○
	<i>S. Montevideo</i>	4	○	○	○	●	○	●	1	○	1	●	1	○	○
O:8(C ₂ -C ₃)	<i>S. Kentucky</i>	19	●	2	○	●	○	●	●	●	●	●	●	●	○
	<i>S. Kottbus</i>	12	●	○	○	●	○	●	●	○	●	●	●	○	1
	<i>S. Blockley</i>	7	●	○	○	●	○	●	●	●	●	●	●	○	●
	<i>S. Bazenheid</i>	5	●	○	○	●	○	●	●	●	●	●	●	○	●
O:9(D ₁)	<i>S. Pullorum</i>	55	●	○	●	●	●	○	●	44	●	○	○	○	○
	<i>S. Enteritidis</i>	39	●	33	38	●	●	○	●	●	●	○	○	○	○
	<i>S. Gallinarum</i>	9	●	○	7	●	●	○	●	●	●	○	1	○	○
O:3,10(E ₁)	<i>S. Anatum</i>	5	●	○	2	●	○	●	○	○	●	●	●	○	○
	<i>S. Meleagridis</i>	1	●	○	○	○	○	●	●	●	●	●	●	○	○
O:1,3,19(E ₄)	<i>S. Senftenberg</i>	2	●	○	○	●	○	●	○	●	●	●	○	○	○
Overall prevalence (%) ^b			98	27	32	92	32	99	51	73	95	93	56	23	12

^a Numbers indicate the prevalence of the gene (percent) in the serovar when the gene was present in some of the strains; otherwise, a filled circle indicates that the gene was present in every strain of the *Salmonella* serovar tested, while an open circle indicates that the gene was not present in any of the strains. Data for *bcfD*, *csgA*, *fimA*, *stdB*, and *sthE* are not shown as they were present in all 323 *Salmonella* strains tested while data for *staA* and *stgA* were omitted as they were not present in any strain.

^b Based on a total of 323 strains.

serogroup D1, and *pefA*, *stkA* and *tcfA* were present in only some isolates of some serovars (Table 3 and Fig. 2). In addition, lack of *stiH* seems to be mainly associated with *S. Indiana*, and all *S. Montevideo* strains lack *lpfD*. Furthermore, *sefA* is nearly exclusively associated with *S. Pullorum*, *S. Gallinarum*, and *S. Enteritidis* strains, and the generally plasmid-encoded *pefA* is mainly associated with *S. Typhimurium* and *S. Enteritidis* strains which contain the *Salmonella* virulence plasmid or other derivative plasmids.

DISCUSSION

S. enterica is the most commonly reported cause of bacterial foodborne illness worldwide and is mainly associated with the ingestion of poultry and its products. We found a high prevalence in the five poultry species we studied in our widespread study involving 51 farms in seven regions of China. The overall prevalence of 9.8% was similar to that recently reported in duck farms in China (12.2% [20]) and other reports, including those describing broiler breeder farms in the United States (6.8% [21]) and broiler flocks in Austria (7.7% [22]). However, higher prevalences of *Salmonella* carriage in poultry farms than in this investigation were also reported in broiler flocks in the Republic of Ireland (27.3% [23]) and on Reunion Island (27% [24]). We found that the prevalence of *Salmonella* serovars from five species of poultry were different, and the main serovars were *S. Pullorum* (chicken), *S. Enteritidis* (duck), *S. Typhimurium* (goose), *S. Typhimurium* (pigeon), and *S. Saintpaul* (turkey). Other studies indicated that the prevalent serovars of *Salmonella* were usually correlated with a specific spe-

cies or region (25, 26). The host specificity was not observed in this study since the serovars of *S. Typhimurium*, *S. Enteritidis*, *S. Heidelberg*, and *S. Kentucky* were isolated from nearly all of these five poultry species. These serovars had been frequently isolated from poultry products in China (20, 27–29), and this indicated a great correspondence between *Salmonella*-contaminated food and salmonellosis.

In this study, *S. Pullorum* was the most prevalent serovar (17.0%) isolated from the five poultry species. This was primarily due to the high proportion (47.8%) of the samples from chicken (*S. Pullorum* was mainly isolated from chicken, with only four strains from turkey), but also verified the high carriage rate of pullorum disease in China. Previous publications showed that pullorum disease infection rates in China were usually over 30% (30), which was different from what has been reported in developed countries (31). For a long time the prevention and control of pullorum disease in China have mainly depended on the use of antibiotics, leading to a high level of drug-resistant bacteria (32), and this made the prevention and control a great challenge. Recently, the official document on the National Medium and Long-Term Planning for Prevention and Control of Animal Epidemics (2012–2020) was issued in China (33), in which the government outlined plans to control pullorum disease through the detection and purification through a medium- or long-term program.

Our study of the distribution of various fimbrial genes in *Salmonella* serovars from poultry in China has shown that over 70% of the isolates possessed the *bcfD*, *csgA*, *fimA*, *stdB*, *sthE*, *stbD*, *lpfD*,

# of fimbrial genes	Serotypes (# of isolates)	Fimbrial genes																			
		<i>bcfD</i>	<i>csgA</i>	<i>fimA</i>	<i>stcB</i>	<i>stfE</i>	<i>stbD</i>	<i>stiH</i>	<i>safC</i>	<i>lpfD</i>	<i>stjA</i>	<i>stcA</i>	<i>stfH</i>	<i>steB</i>	<i>stkA</i>	<i>tcfA</i>	<i>pegD</i>	<i>sefA</i>	<i>pefA</i>	<i>staA</i>	<i>stgA</i>
8	Montevideo (2)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
9	Reading (3)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Montevideo (1)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
10	Montevideo (1)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
11	Thompson (16)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Anatum (3)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
12	Indiana (1)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Indiana (2)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
13	Indiana (2)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Senftenberg(2), Derby(1)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
14	Derby (2)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Gallinarum (2)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
15	Indiana (5)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Ko (11), Sa (10), Tm (5)*	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
16	Anatum (2)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Typhimurium (1)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
17	Meleagridis (1)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Indiana (7)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
18	Indiana (3)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Indiana (2)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
19	Indiana (3)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Typhimurium (3)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
20	Pullorum (11)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Potsdam (3)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
21	Potsdam (16), Agona(10)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Ga (6), En (6), Pu (44)*	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
22	Enteritidis (1)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Saintpaul (3), Tm (36)*	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
23	Kottbus (1)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Heidelberg (3)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
24	Heidelberg (2)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Heidelberg (4)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
25	Potsdam (3)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Kentucky (17)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
26	Bazenheid (5), Bl (7)*	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Enteritidis (32)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
27	Gallinarum (1)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Typhimurium (5)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
28	Heidelberg (9)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Kentucky (2)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
29	Heidelberg (5)	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

FIG 2 Distribution of *Salmonella* fimbrial genes in 20 serovars investigated in this study. On the left, 20 *Salmonella* serotypes are ordered from top to bottom based on the number of fimbrial genes (8, 9, 10, 11, 12, 13, 14, or 15) in the *Salmonella* isolates. The top of this figure gives the names of 20 fimbrial genes investigated in this study. A filled box denotes the presence of the fimbrial gene, and an open box indicates the absence of the gene. Where space was insufficient to list all serovars, some were abbreviated, as indicated by an asterisk: Ko, Kottbus; Sa Saintpaul; Tm, Typhimurium; Ga, Gallinarum; En, Enteritidis; Pu, Pullorum; and Bl, Blockley.

stfH, *stiH*, *safC*, and *steB* genes, which is consistent with previous studies (10, 34) and provides further evidence that these fimbrial genes are widely distributed among *S. enterica*. We found lower prevalences of *tcfA*, *stkA*, *pefA*, *sefA*, *pegD*, *stcA*, and *stjA* (11.8 to 55.7%) and no evidence of *staA* and *stgA*, two genes which were once reported in *S. Pullorum* from poultry, suggesting that some fimbrial genes might be serovar specific (10, 12). Information about the presence or absence of specific fimbrial genes in certain *Salmonella* serovars may provide potential value as the fimbrial genotype could be used to predict certain *Salmonella* serotypes.

Previous studies showed that *sef* and *sta* FGCs exist only in serogroup D1 and *S. Typhi* (12, 35), and our study was consistent

with these reports. Although we found in this investigation the association of *safC*, *steB*, *stjA*, *stcA*, *pegD*, *pefA*, *stkA*, and *tcfA* in our isolates with certain serovars or serotypes, determination of their definitive correlations will require further studies with larger numbers of isolates from a wider source of animals. The accumulation of pseudogenes is a key feature of *Salmonella* and other host-adapted pathogens, and overlapping pseudogene complements are evident in many *Salmonella* serovars (36). In this investigation, the analysis of one fimbrial gene per cluster by PCR (rather than the cloning of each full-length gene) cannot give complete information about the host specificity, pathogenicity, and transmission efficiency of each *Salmonella* phenotype. The

full length of each gene should be sequenced in the future to verify the conclusions of this study.

Investigating the differential distribution of fimbrial genes will help create a novel strategy for genotyping *Salmonella* strains, and knowledge of the correlation between fimbrial genotypes and serotypes will further help investigations of the mutation and evolution of *Salmonella* strains. The conclusions of this study may lead to a further understanding of the genetic evolution, strain virulence, disease progression, transmission efficiency, and host range of poultry *Salmonella* and provide support for the development of new approaches for the control of *Salmonella* infection.

ACKNOWLEDGMENTS

This project was supported by grants from the National Natural Science Foundation of China (31101833 and 31272575), the Special Fund for Agroscientific Research in the Public Interest (201303044), the Personnel Developing Program of Jiangsu Province (NY-024), and the Scientific and Technological Project of Yangzhou (2011091 and 2012080).

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