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Prevalence and molecular characterization of *Salmonella enterica* isolates throughout an integrated broiler supply chain in China

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SUMMARY

A total of 1145 samples were collected from chicken breeder farms, hatcheries, broiler farms, a slaughterhouse and retail refrigerated chicken stores in an integrated broiler supply chain in Guangdong Province, China, in 2013. One-hundred and two *Salmonella enterica* strains were isolated and subjected to serotyping, antimicrobial susceptibility testing, virulence profile determination and molecular subtyping by pulsed field gel electrophoresis (PFGE). The contamination rates in samples from breeder farms, hatcheries, broiler farms, the slaughterhouse and retail stores were 1·46%, 4·31%, 7·00%, 62·86% and 54·67%, respectively. The isolated strains of *S. enterica* belonged to 10 serotypes; most of them were *S.* Weltevreden (46·08%, 47/102) and *S.* Agona (18·63%, 19/102). Isolates were frequently resistant to streptomycin (38·2%), tetracycline (36·3%), sulfisoxazole (35·3%) and gentamicin (34·3%); 31·4% of isolates were multidrug resistant. The isolates were screened for 10 virulence factors. The *Salmonella* pathogenicity island genes *avrA, ssaQ, mgtC, siiD*, and *sopB* and the fimbrial gene *bcfC* were present in 100% of the strains. PFGE genotyping of the 102 *S. enterica* isolates yielded 24 PFGE types at an 85% similarity threshold. The PFGE patterns show that the genotypes of *S. enterica* in the production chain are very diverse, but some strains have 100% similarity in different parts of the production chain, which indicates that some *S. enterica* persist throughout the broiler supply chain.

Key words: Antimicrobial resistance, broiler supply chain, PFGE, *Salmonella enterica*, virulence.

INTRODUCTION

Salmonellosis is a worldwide foodborne illness. The CDC estimates that ~1·2 million illnesses and 450 deaths occur annually due to non-typhoidal *Salmonella* in the United States [1]. In China, analyst estimates suggest that foodborne *Salmonella* causes 8·2 million cases of diarrhoea and 792 deaths per year [2]. Outbreaks and sporadic illness of *Salmonella enterica* infections have been associated with ingestion of contaminated foods, including animal origin, fruits and vegetables [3].

Food of animal origin, especially poultry and poultry products, is considered a major reservoir for many serotypes of *S. enterica*, and human infection is often attributed to consumption of contaminated poultry products such as eggs and chicken [4]. Contamination of chicken or chicken meat may occur throughout the whole production chain and some important risk

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factors for contamination at each stage of this process have been identified. The risk factors include inadequate cleaning and disinfection of broiler rearing houses, contamination of feed, and chicken production process; these factors can be a significant source of pathogen contamination between carcasses [5]. To our knowledge, *S. enterica* always exists in the alimentary tract and reproductive system of carrier chickens. Studies have demonstrated that *S. enterica* can be spread from one broiler breeding flock to seven broiler flocks and further to the abattoir, and can therefore be transmitted to humans through contaminated chicken production [6]. Reduction or elimination of *S. enterica* in broiler supply chains, particularly in chicken products, is important to control this disease, and insights into the occurrence of *S. enterica* and factors affecting its prevalence are essential [7].

Chinese broiler production ranks second in the world; ~28 million tons of chicken are consumed in China each year [8]. As one of the largest chicken-consumption countries around the world, great attention should be paid to the dangers of *S. enterica* in chicken via monitoring the prevalence of *S. enterica* in whole broiler supply chain in China. However, the dissemination of *S. enterica* through an integrated broiler supply chain has not previously been studied in China. Therefore, investigation of the prevalence of *S. enterica* in a broiler supply chain will provide valuable information for the effective prevention of salmonellosis.

The aims of this study were to evaluate the prevalence of *S. enterica* throughout a broiler supply chain in China, from breeder farms through to retail stores, and to characterize the *S. enterica* isolates from that broiler supply chain by determining the genetic relationships, virulence profiles and antimicrobial resistance patterns of the strains.

MATERIAL AND METHODS

Sample collection

From July 2013 to December 2013, we chose a single integration broiler supply chain to perform the sample collection. The supply chain consisted of two breeder farms and its two downstream chicken hatcheries, two broiler farms, one slaughterhouse and five raw chicken retail stores in Guangdong Province, China. The production process was as follows, two breeder farms randomly supplied hatching eggs to two hatcheries, and the eggs were hatched there for 21 days. Thereafter, the 1-day-old chicks were transferred randomly to two broiler farms, after which the 6- to 7-week-old broilers were transported to a single slaughterhouse for processing. Slaughtered broilers were sent to the subordinate five retail stores or other food enterprises.

In this study, a total of 1145 samples were collected at five different stages of the broiler supply chain including breeder farms ($n = 480$), hatcheries ($n = 255$), broiler farms ($n = 300$), the slaughterhouse ($n = 35$) and retail stores ($n = 75$). Samples from the chicken farms were collected with sterile cloacal swabs, and samples from the slaughterhouse and retail stores were collected by washing intact whole chicken carcasses. Rectal swabs were taken randomly from individual healthy birds as described previously [9]. The sampling was divided into three stages, with each stage lasting 2 months. Monthly, every site (farm, slaughterhouse or store) in each stage would randomly collect samples on two occasions. In the first stage, samples were taken from the breeder farm and hatchery; 50–70 samples were collected from a breeder farm once, and 30–40 samples were collected from a hatchery each time. In the second stage, 30–40 samples were taken from a broiler farm each time. In the third stage, samples were taken from the slaughterhouse and retail store, with 5–10 collected from the slaughterhouse each time and 10–15 from the retail store once.

Isolation, identification, and serotyping of *S. enterica*

S. enterica isolation was performed as described previously [10, 11]. Briefly, swabs were cultured in 9 ml selenite cystine broth (Difco, USA) at 37 °C for 24 h, then 100 μl aliquots of the broth were streaked onto xylose lysine deoxycholate (XLD; Difco) plates and incubated at 37 °C for 24 h, typical *Salmonella* colonies were seen. They were further identified using API identification kits (bioMérieux, France) and serotyped with commercial antiserum (S&A Reagents Laboratory, Thailand). Serotype was assigned according to the Kauffmann–White scheme [12]. For chicken carcasses, a whole piece was placed in a plastic bag and washed with 400 ml buffered peptone water (Difco) 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 tetrathionate (TT; Difco) and Rappaport–Vassiliadis (RV; Difco) broth incubated at 42 °C for 24 h, respectively, followed by streaking from TT onto xylose lysine tergitol 4 (Difco) agar and from RV onto XLD agar incubated at 37 °C for 24 h, presumptive *Salmonella*

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colonies were seen. The following identification and serotyping were performed as described above.

Antimicrobial susceptibility testing

A total of 20 antimicrobial agents currently used in veterinary and medical therapy were assessed according to the Kirby–Bauer disk diffusion method by using antibiotic discs (Oxoid, UK) [13]. *S. enterica* isolates were tested with ampicillin (10 μg), amoxicillin (20 μg), ceftriaxone (30 μg), cefoperazone (75 μg), cefepime (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), imipenem (10 μg), gentamicin (10 μg), amikacin (30 μg), kanamycin (30 μg), streptomycin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), trimethoprim/sulfamethoxazole (1·25/23·75 μg), trimethoprim (5μg), and sulfisoxazole (300 μg). *Escherichia coli* strain ATCC 25922 was used as a quality control organism. The classes of resistance level were defined as described by the Clinical and Laboratory Standards Institute and are indicated as susceptible (S), intermediate (I) or resistant (R) [14].

Polymerase chain reaction (PCR) detection of virulence genes

All isolates of *S. enterica* were screened for 10 virulence genes by a PCR method. Primers used in this study are listed in Table 1. Virulence determinants for each strain analysed were categorized according to their location on the *Salmonella* genome: *Salmonella* pathogenicity islands (SPIs) (*avrA, ssaQ, mgtC, siiD*, and *sopB*), prophages (*gipA, sodC1*, and *sopE*), a plasmid (*spvC*), and a fimbrial cluster (*bcfC*). Genomic DNA was isolated with a Bacterial Genomic DNA kit (Omega, USA) according to the manufacturer's instructions. The PCR cycling conditions were: 5 min at 95 °C; 30 cycles of 40 s at 94 °C, 60 s at 55 °C, and 90 s at 72 °C; with a final extension of 10 min at 72 °C. The PCR products were analysed by electrophoresis and visualized under ultraviolet light.

Pulsed-field gel electrophoresis (PFGE) analysis

PFGE of the 102 *S. enterica* isolates was performed using the PulseNet standardized protocol [15]. Digested DNA was separated using a Chef Mapper (Bio-Rad, USA), and the gels were stained with Lonza GelStar Nucleic Acid Gel Stain (Cambrex Bio Science, USA) and analysed using BioNumerics software (Applied Maths, Belgium). Briefly, agarose-embedded DNA was digested with 50 U *Xba*I (TaKaRa, China) for 1·5–2 h in a water bath at 37 °C. Restriction fragments were separated by electrophoresis in 0·5× TBE buffer at 14 °C for 18 h using a Chef Mapper electrophoresis system with pulse times of 2·16–63·8 s. *S. enterica* serotype Braenderup H9812 was used as the molecular weight size standard.

RESULTS

Isolation and serotype distribution of *S. enterica*

Of the 1145 collected samples, 102 were positive for *S. enterica*, including seven (1·46%) of the 480 breeder farm samples, 11 (4·31%) of the 255 hatchery samples, 21 (7·00%) of the 300 broiler farm samples, 22 (62·86%) of the 35 slaughterhouse samples and 41 (54·67%) of the 75 retail store raw chicken samples. Serotyping results revealed the presence of 10 different serotypes with *S.* Weltevreden ($n = 47$) being dominant, accounting for 46·08% of the isolates, followed by *S.* Agona ($n = 19$, 18·63%), *S.* Meleagridis ($n = 15$, 14·71%), *S.* Enteritidis ($n = 8$, 7·84%) and other serotypes (Table 2).

Antimicrobial resistance in *S. enterica* strains

Overall, 73 (71·5%) of the 102 strains analysed were resistant to at least one antimicrobial drug of the 20 tested (Table 3). The most commonly observed resistances were to streptomycin (38·2%), tetracycline (36·3%), sulfisoxazole (35·3%) and gentamicin (34·3%). All isolates were susceptible to cefepime, ceftazidime, imipenem, and ofloxacin. The strains isolated from the breeder farms were the most sensitive to antibiotics; the highest resistance was to streptomycin (28·6%). From hatcheries, isolates were most commonly resistant to nalidixic acid (72·7%) and sulfisoxazole (72·7%). The stains isolated from broiler farms, the slaughterhouse and retail stores were most resistant to aminoglycosides, including gentamicin (66·7%), gentamicin (36·4%) and streptomycin (48·8%), respectively.

Of the 53 isolates (52·0%) that showed resistance to $\geqslant$2 antimicrobials, 32 (31·4%) exhibited varying degrees of multidrug resistance (MDR), defined as resistance to $\geqslant$3 different classes of antimicrobials. These strains included four different serotypes and

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Table 1. *Primers for amplification of virulence genes in this study*

Gene	Location	Function	Primer (5′–3′)	PCR product size (bp)	Ref.
avrA	SPI 1	Inhibits the key pro-inflammatory, anti-apoptotic NF-kappa B pathway	GTTATGGACGGAACGACATCGG ATTCTGCTTCCCGCCGCC	385	[46]
ssaQ	SPI 2	Secretion system apparatus protein, component of second T3SS	GAATAGCGAATGAAGAGCGTCC CATCGTGTTATCCTCTGTCAGC	677	[47]
mgtC	SPI 3	Intra-macrophage survival protein	TGACTATCAATGCTCCAGTGAAT ATTTACTGGCCGCTATGCTGTTG	655	[47]
siiD	SPI 4	HLYD family secretion protein	GAATAGAAGACAAAGCGATCATC GCTTTGTCCACGCCTTTCATC	1231	[47]
sopB	SPI 5	Translocated effector protein via T3SS	GATGTGATTAATGAAGAAATGCC GCAAACCATAAAAACTACACTCA	1170	[47]
gipA	Gifsy-1 bacteriophage	Peyer's patch-specific virulence factor	GCAAGCTGTACATGGCAAAG GGTATCGGTGACGAACAAAT	212	[48]
sodC1	Gifsy-2 bacteriophage	Periplasmic Cu, Zn-superoxide dismutases	CCAGTGGAGCAGGTTTATCG GGTGCGCTCATCAGTTGTTC	424	[49]
sopE	SopEPhi bacteriophage	Translocated T3SS effector protein	ACACACTTTCACCGAGGAAGCG GGATGCCTTCTGATGTTGACTGG	398	[46]
spvC	pSLT plasmid	Growth and survival within host	ACTCCTTGCACAACCAAATGCGGA TGTCTTCTGCATTTCGCCACCATCA	570	[50]
bcfC	Chromosome	Bovine colonization factor, fimbrial usher	ACCAGAGACATTGCCTTCC TTCTGATCGCCGCTATTCG	467	[51]

Table 2. *Serotype distribution of* Salmonella enterica *in the integrated broiler supply chain*

	Source of sample						
Serotype	Breeder farms	Hatcheries	Broiler farms	Slaughterhouse	Retail stores	No.	%
Weltevreden	1	0	17	15	14	47	46·08
Agona	1	0	4	7	7	19	18·63
Meleagridis	0	0	0	0	15	15	14·71
Enteritidis	0	8	0	0	0	8	7·84
Thompson	0	3	0	0	1	4	3·92
Senftenberg	3	0	0	0	0	3	2·94
Derby	0	0	0	0	3	3	2·94
Stanley	1	0	0	0	0	1	0·98
Infantis	1	0	0	0	0	1	0·98
Rissen	0	0	0	0	1	1	0·98
Total	7	11	21	22	41	102	100

14 distinct MDR patterns: *S.* Weltevreden (23 isolates with eight different MDR patterns), *S.* Enteritidis (seven isolates with four different MDR patterns), *S.* Infantis (one isolate) and *S.* Rissen (one isolate). The MDR pattern most frequently observed was STR-GEN-KAN-TET-SUL-SXT-TMP-AMP-AMX-CHL (46·9%), which was found in *S.* Weltevreden (Table 4).

Detection of virulence genes

All the *S. enterica* isolates were positive in PCR tests for the SPI genes *avrA, ssaQ, mgtC, siiD*, and *sopB*, and the fimbrial gene *bcfC*. Lower prevalences were observed for *sopE, sodC1, gipA* and *spvC* genes (60%, 57%, 51% and 8%, respectively). The plasmid-borne virulence gene *spvC* was only found in serovar *S.* Enteritidis

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Table 3. *Percentages of* Salmonella enterica *isolates from the integrated broiler supply chain resistant to each antimicrobial*

	Number of resistant isolates (%) from					
Antibiotic	Breeder farms ($n = 7$)	Hatcheries ($n = 11$)	Broiler farms ($n = 21$)	Slaughterhouse ($n = 22$)	Retail stores ($n = 41$)	Total ($n = 102$)
β-lactams						
Ampicillin	1 (14·3)	6 (54·5)	6 (28·6)	5 (22·7)	13 (31·7)	31 (30·4)
Amoxicillin	1 (14·3)	6 (54·5)	6 (28·6)	5 (22·7)	13 (31·7)	31 (30·4)
Ceftriaxone	0	0	0	0	0	0
Cefoperazone	0	0	2 (9·3)	1 (4·6)	0	3 (3·0)
Cefepime	0	0	0	0	0	0
Cefotaxime	0	0	0	0	0	0
Ceftazidime	0	0	0	0	0	0
Imipenem	0	0	0	0	0	0
Aminoglycosides						
Gentamicin	1 (14·3)	0	14 (66·7)	8 (36·4)	12 (29·3)	35 (34·3)
Amikacin	0	0	11 (52·4)	8 (36·4)	2 (4·3)	21 (20·6)
Kanamycin	0	0	12 (57·1)	8 (36·4)	14 (34·2)	34 (33·3)
Streptomycin	2 (28·6)	5 (45·5)	7 (33·3)	5 (22·7)	20 (48·8)	39 (38·2)
Tetracyclines						
Tetracycline	1 (14·3)	3 (27·3)	8 (38·1)	7 (31·8)	18 (43·9)	37 (36·3)
Phenicols						
Chloramphenicol	1 (14·3)	0	7 (33·3)	1 (4·6)	13 (31·7)	22 (21·6)
Quinolones and fluoroquinolones						
Nalidixic acid	1 (14·3)	8 (72·7)	2 (9·5)	0	0	11 (10·8)
Ciprofloxacin	0	0	3 (14·3)	0	0	3 (2·9)
Ofloxacin	0	0	0	0	0	0
Sulfonamides and synergistic agents						
Trimethoprim/sulfamethoxazole	1 (14·3)	1 (9·1)	5 (23·8)	1 (4·6)	15 (36·6)	23 (22·6)
Trimethoprim	1 (14·3)	1 (9·1)	5 (23·8)	1 (4·6)	15 (36·6)	23 (22·6)
Sulfisoxazole	1 (14·3)	8 (72·7)	7 (33·3)	5 (22·7)	15 (36·6)	36 (35·3)

isolates. Virulence profiles *of S. enterica* strains showed concordance with serotyping; Table 5 shows the data.

PFGE analysis

At an 85% pattern similarity threshold, PFGE patterns from the 102 *S. enterica* isolates were grouped into 24 clusters (A–X) (Fig. 1). These clusters showed concordance with serotypes, antibiotic resistance profiles and virulence profiles. The clusters included *S.* Weltevreden ($n = 47$, clusters E–L), *S.* Agona ($n = 19$, V–X), *S.* Meleagridis ($n = 15$, Q–T), *S.* Enteritidis ($n = 8$, P), *S.* Thompson ($n = 4$, N–O), *S.* Senftenberg ($n = 3$, A–B), *S.* Derby ($n = 3$, U), *S.* Stanley ($n = 1$, M), *S.* Infantis ($n = 1$, C), and *S.* Rissen ($n = 1$, D). In the eight *S.* Weltevreden isolate clusters, E, G and J were the largest and comprised 70·21% ($n = 33$) of the total number of *S.* Weltevreden isolates. Cluster E included broiler farm, slaughterhouse and retail store isolates. Cluster G contained 12 isolates which originated only from the slaughterhouse. Cluster J contained nine isolates, eight of which originated from retail stores and one from a broiler farm. The *S.* Agona isolates formed three gene clusters, of which cluster X was the largest; it included broiler farm, slaughterhouse and retail store isolates. Furthermore, the broiler farm isolates SA038, SA019, and SA031 had the same patterns, respectively, as the retail store isolates SA087, SA072, and SA085. The breeder farm isolate SA007 had the same pattern as the retail store isolate SA078. The detection of common PFGE profiles indicates that isolates of *S. enterica* were present throughout the integrated broiler supply chain and have the potential for transmission from poultry products to humans.

DISCUSSION

We collected a total of 1145 samples from breeder farms through to retail refrigerated chicken stores in

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Table 4. *Multidrug resistance profiles of* Salmonella enterica *isolates from the integrated broiler supply chain*

Resistotype	Multiple-resistance pattern	Source (no. of isolates)	Serovar	No.
R01	STR-GEN-KAN-AMK-TET-SUL-SXT-TMP-AMP-AMX-CHL-NAL-CIP	Broiler farm (1)	Weltevreden	1
R02	STR-GEN-KAN-TET-SUL-SXT-TMP-AMP-AMX-CHL	Slaughterhouse (1), broiler farms (2), retail stores (12)	Weltevreden	15
R03	STR-GEN-KAN-AMK-TET-SUL-AMP-AMX-CFP	Broiler farm (1)	Weltevreden	1
R04	STR-GEN-TET-SUL-SXT-TMP-AMP-AMX-CHL	Broiler farm (1)	Weltevreden	1
R05	STR-GEN-KAN-AMK-TET-SUL-AMP-AMX	Slaughterhouse (2)	Weltevreden	2
R06	STR-GEN-KAN-TET-SUL-AMP-AMX-CFP	Slaughterhouse (1)	Weltevreden	1
R07	STR-GEN-TET-SUL-SXT-TMP-CHL	Broiler farm (1)	Weltevreden	1
R08	STR-GEN-KAN-TET-SUL-AMP-AMX	Slaughterhouse (1)	Weltevreden	1
R09	STR-TET-SUL-SXT-TMP-NAL	Hatchery (1)	Enteritidis	1
R10	STR-TET-SUL-AMP-AMX-NAL	Hatchery (1)	Enteritidis	1
R11	STR-SUL-AMP-AMX-NAL	Hatchery (1)	Enteritidis	1
R12	SUL-AMP-AMX-NAL	Hatcheries (4)	Enteritidis	4
R13	STR-SUL-SXT-TMP-AMP-AMX-CHL-NAL	Breeder farm (1)	Infantis	1
R14	TET-SUL-SXT-TMP-AMP-AMX	Retail store (1)	Rissen	1

AMK, Amikacin; AMP, ampicillin; AMX, amoxicillin; CAZ, ceftazidime; CFP, cefoperazone; CHL, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; FEP, cefepime; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; SUL, sulfisoxazole; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TMP, trimethoprim.

Table 5. *Virulence profiles of strains of the various* Salmonella enterica *serovar isolates from the integrated broiler supply chain*

	Virulence gene										
Serotype	*avrA*	*ssaQ*	*mgtC*	*siiD*	*sopB*	*gipA*	*sodC1*	*sopE*	*spvC*	*bcfC*	No. of strains
Salmonella Weltevreden	■	■	■	■	■	■	■	■	□	■	29
	■	■	■	■	■	■	■	□	□	■	18
Salmonella Agona	■	■	■	■	■	■	□	■	□	■	2
	■	■	■	■	■	□	□	■	□	■	17
Salmonella Meleagridis	■	■	■	■	■	□	□	■	□	■	1
	■	■	■	■	■	□	□	□	□	■	14
Salmonella Enteritidis	■	■	■	■	■	□	■	■	■	■	8
Salmonella Thompson	■	■	■	■	■	□	□	□	□	■	4
Salmonella Senftenberg	■	■	■	■	■	■	□	□	□	■	3
Salmonella Derby	■	■	■	■	■	□	■	■	□	■	3
Salmonella Stanley	■	■	■	■	■	□	□	■	□	■	1
Salmonella Infantis	■	■	■	■	■	□	□	□	□	■	1
Salmonella Rissen	■	■	■	■	■	□	□	□	□	■	1
Prevalence (%) in the 102 strains	100%	100%	100%	100%	100%	51%	57%	60%	8%	100%	

■, Indicates the designated virulence gene was present in every strain of the *S. enterica* serovar tested.
□, Indicates the designated virulence gene was not present in any strain of the *S. enterica* serovar tested.

Guangdong, China, in 2013, to assess the prevalence of *S. enterica* in a single integrated broiler supply chain; 102 *S. enterica* strains were isolated. The *S. enterica* contamination rate significantly increased from breeder farm to slaughterhouse, similar to reports from The Netherlands and the United States [16, 17]. In the present study, 1·46% of the breeder farm samples were contaminated with *S. enterica*, compared to 5·5% in 2012 in Shandong Province (China) [18] and 6·8% in the United States [19]; the

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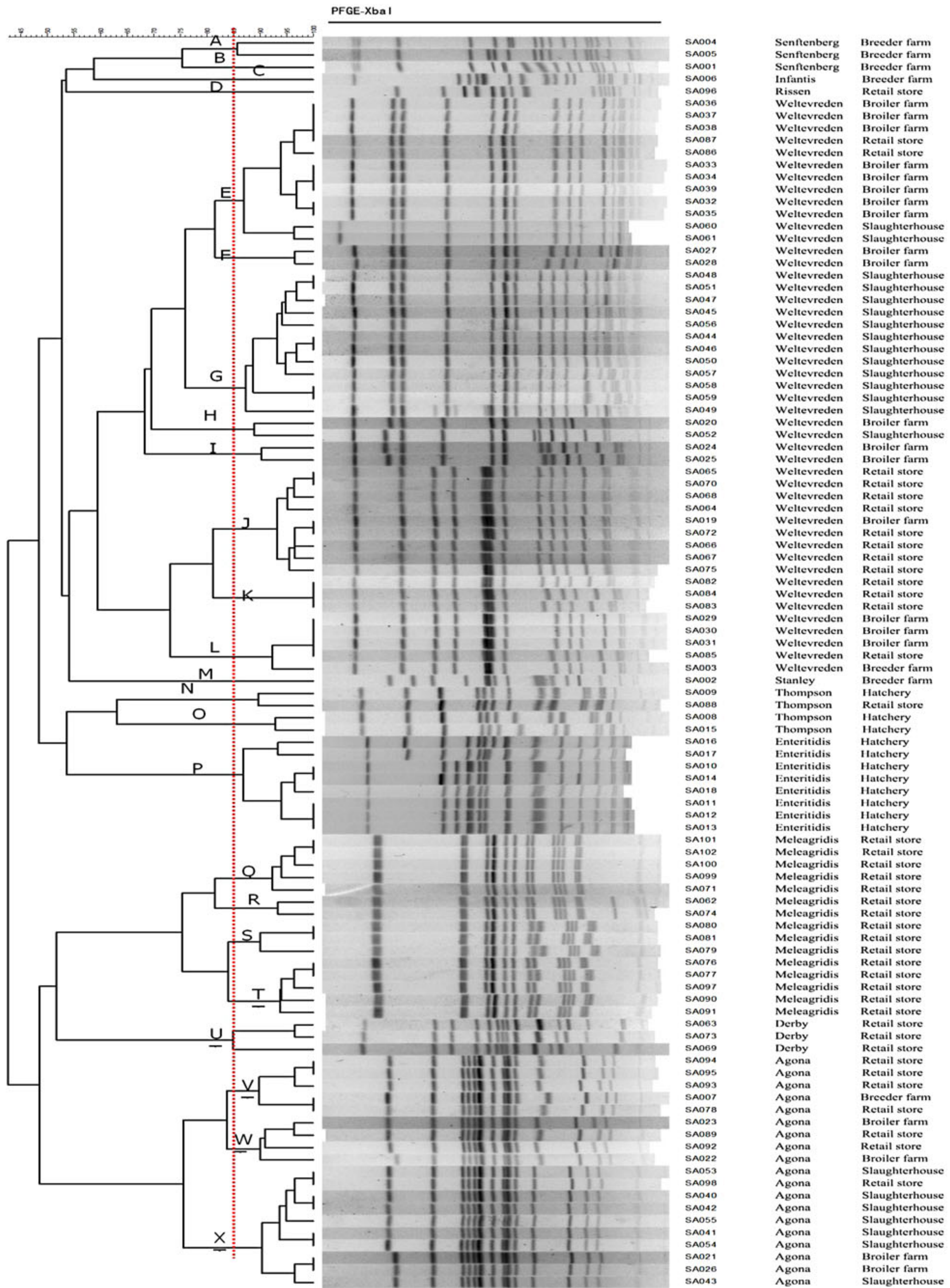

Fig. 1. *Xba*I pulsed-field gel electrophoresis patterns of 102 *Salmonella* isolates in this study.

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isolation rate from broiler farm samples was low. The *S. enterica* isolation rate of 4·31% from hatchery samples and 7·00% from broiler farm samples was also lower than that in other provinces of China and in other countries [20–22]. However, the *S. enterica* contamination rate in the slaughterhouse (62·86%) and retail raw chicken stores (54·67%) was higher than those reported in other provinces of China and in Western countries [23–25]. The high contamination rates in the consumer part of the supply chain show that chicken products are likely to be an important vector of *S. enterica*, and there may be a significant hygienic problem in this stages. Previous studies have shown that continuous 'silent' circulation of *S. enterica* in the broiler supply system poses a potential risk of spread of *S. enterica* and spillover to humans [26, 27]. To deal with high contamination rates in slaughterhouses and retail markets, better management and improved hygiene are required in these environments, such as reinforcing safeguards, disinfecting strictly, and an all-in/all-out operation. Improved hygiene management during transport of broilers can significantly reduce the risk of *S. enterica* contamination of poultry meat [28]. In addition, vaccine has been widely used in the prevention and treatment of infectious diseases, universal vaccination may be such a strategy to prevent the prevalence of *S. enterica* in the farm, but there is no commercial *Salmonella* vaccine in China.

The serotype distribution varied significantly throughout the supply chain, although *S. enterica* serotypes Weltevreden and Agona were the most common serotypes, and were isolated from breeder farms, broiler farms, the slaughterhouse and retail stores. A previous study indicated that the distribution of *S. enterica* serotypes in poultry may change geographically and temporally, and *S.* Enteritidis, *S.* Typhimurium and *S.* Pullorum were the three most common serotypes in China (2006–2012) [29]. The reason for this difference from our study may be that we only investigated a single production chain. *S*. Weltevreden is one of the top ten serotypes implicated in human disease in Guangdong Province [30]. It has long been a major problem associated with meat products in South-East Asia and was also reported as one of the major serotypes in humans in Thailand [31, 32]. *S.* Weltevreden is an emerging serotype associated with meat, seafood and plant products in Western countries [33, 34]. In China, especially in the south, the combination of urban consumer groups with large consumption of seafood and poultry products suggests that government should strengthen monitoring and prevention strategies for *S.* Weltevreden.

S. enterica isolates exhibited resistance to a wide spectrum of antimicrobials including streptomycin (38·2%), tetracycline (36·3%), sulfisoxazole (35·3%) and gentamicin (34·3%). These findings agree with previous reports on antibiotic resistance in chickens [23, 35]. One potential explanation is that sulfonamides have been used for 30 years in human and veterinary medicine [36], while tetracyclines and aminoglycosides have long been used as feed additives for therapy, prophylaxis and growth promotion in broiler production. Previous studies have reported a correlation between the use of feed supplemented with antibiotics and the development of MDR in Enterobacteriaceae [37, 38]. Conversely, low levels of resistance to cephalosporins were observed throughout our study, which may be due to the limited use of this class of antimicrobial agents in veterinary medicine in China.

The different antibiotic resistances observed in the supply chain may be the result of the different types and amounts of antibiotic use in each link of the chain. Isolates from breeder farms did not have severe antibiotic resistance, whereas the *S. enterica* isolated from broiler farms had high resistance to antibiotics, which may be a result of different management of biosecurity and hygiene. Breeder farm, broiler farm and hatchery *S. enterica* serotype isolates are different from each other, which might also be the reason for different antibiotic resistances [11]. In our research, 67·65% (69/102) of the isolated strains were resistant to at least one kind of antibiotic, while 48·04% of the strains had $\geqslant 3$ types of antibiotic resistance, including some isolates resistant to $\geqslant 10$ drugs. Such resistance can be passed through the food chain to the human population [39]. Eventually it can lead to microbial cross-resistance and a threat to human health [40].

In the present study, ten potential virulence factor genes were investigated. The virulence genes showing the highest prevalence (100%) were *avrA, ssaQ, mgtC, siiD, sopB* and *bcfC*, which is consistent with a recent study [41]. Our results indicated that pathogenicity island (SPI1-5) and fimbrial genes have high genetic stability. SPIs play a key role in *Salmonella* pathogenesis [42]. Fimbriae help *Salmonella* strains adhere to animal cells and promote their colonization; pretreatment of bacteria with antibodies specific to types 1 and 3 fimbriae increased mouse survival by

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over 60% [43]. *spvC* was only found in serovar *S.* Enteritidis. The prevalence of *gipA, sodC1* and *sopE* genes varied in different serotypes, but they were common in *S.* Weltevreden. Bacteriophages and plasmids can horizontally transfer virulence genes [44]. In certain conditions, there is the possibility of *S. enterica* strains acquiring virulence, which poses a risk to consumers of contaminated food.

PFGE is the current easy and effective method to assess relatedness in *Salmonella* isolates from different sources [45]. We used PFGE to determine whether serotype identification by classical serotyping matched the serotypes predicted based on a comparison of PFGE types; the two datasets showed agreement. We deduce that PFGE agrees with serotypes, antibiotic resistance profiles and virulence profiles. PFGE fingerprinting shows several retail store and slaughterhouse isolates that clustered with breeder farm and broiler farm isolates. Cluster E, the largest PFGE cluster, consisted of isolates from breeder farm, broiler farm, slaughterhouse, and retail store samples, suggesting a possible association between the isolates. A number of isolates from breeder farms, broiler farms, the slaughterhouse and retail stores had common PFGE patterns, suggesting that many MDR and highly virulent strains are transmitted downwards between stages of the production process. The 41 strains of *S. enterica* isolated from raw chicken retail stores can be divided into 16 PFGE patterns, indicating that *S. enterica* contamination from retail store sources is diverse and complicated.

Some highly consistent PFGE patterns from different sources, demonstrated that the propagation phenomena of *S. enterica* clones through the broiler supply chain is associated with the spread of resistance genes and virulence genes. Whereas many PFGE patterns were seen only in a single source, especially in the slaughter and marketing stages, these results suggest that slaughterhouses and retail stores are two crucial points of *S. enterica* contamination and cross-contamination in the broiler supply chain.

In conclusion, this study evaluated the prevalence of *S. enterica* in an integrated broiler supply chain in Guangdong, China. Our findings demonstrate that *S. enterica* was transferred along the broiler supply chain and from the breeder farm to retail stores. However, the flock or carcass cross-contamination of *S. enterica* at one independent stage may be the determinative factor for contamination. The slaughterhouse and retail raw chicken store stages were the crucial points for *S. enterica* contamination. Furthermore, *S.* Weltevreden was the most prominent serotype and persisted throughout the broiler supply chain. Many *S. enterica* isolates showed resistance to multiple antibiotics and high levels of virulence genes, increasing the need for the implementation of control measures to reduce the spread of antimicrobial resistance and virulence. This study will help strengthen both the understanding and epidemiological surveillance of *S. enterica*.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Scallan E, *et al.*** Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases* 2011; **17**: 7–15.
2. **Mao XD, Jun-Feng HU.** Estimation on disease burden of foodborne non-typhoid salmonellosis in China using literature review method. *Chinese Journal of Disease Control & Prevention* 2011; **15**: 622–625.
3. **Jackson BR, *et al.*** Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998–2008. *Emerging Infectious Diseases* 2013; **19**:1239–1244.
4. **Foley SL, *et al.*** Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *Applied and Environmental Microbiology* 2011; **77**: 4273–4279.
5. **Löfström C, *et al.*** Outbreak of *Salmonella enterica* serovar Typhimurium phage type DT41 in Danish poultry production. *Veterinary Microbiology* 2015; **178**: 167–172.
6. **Chao MR, *et al.*** Assessing the prevalence of *Salmonella enterica* in poultry hatcheries by using hatched eggshell membranes. *Poultry Science* 2007; **86**: 1651–1655.
7. **Logue CM, *et al.*** The incidence of antimicrobial-resistant *Salmonella* spp. on freshly processed poultry from US Midwestern processing plants. *Journal of Applied Microbiology* 2003; **94**: 16–24.
8. **IATP.** Institute for Agriculture and Trade Policy 2014. Fair or fowl? Industrialization of poultry production in China (http://www.iatp.org/files/2014_02_25_PoultryReport_f_web.pdf).
9. **Visscher CF, *et al.*** Serodiversity and serological as well as cultural distribution of *Salmonella* on farms and in

https://doi.org/10.1017/S0950268816001515 Published online by Cambridge University Press

abattoirs in Lower Saxony, Germany. *International Journal of Food Microbiology* 2011; **146**: 44–51.
10. **Gong J, *et al.*** Prevalence and fimbrial genotype distribution of poultry *Salmonella* isolates in China (2006 to 2012). *Applied Environmental Microbiology* 2014; **80**: 687–693.
11. **Yang B, *et al.*** Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. *International Journal of Food Microbiology* 2010; **141**: 63–72.
12. **Popoff MY, *et al.*** Supplement 2002 (no. 46) to 417 the Kauffmann–White scheme. *Research in Microbiology* 2004; **155**: 568–570.
13. **CLSI.** Performance standards for antimicrobial disk susceptibility tests, CLSI document M02-A10. Clinical and Laboratory Standards Institute, 2009.
14. **CLSI.** Performance standards for antimicrobial susceptibility testing; 20th informational supplement, CLSI document M100-S20. Clinical and Laboratory Standards Institute, 2010.
15. **Ribot EM, *et al.*** Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathogens and Disease* 2006; **3**: 59–67.
16. **Van Der Fels-Klerx HJ, *et al.*** Prevalence of *Salmonella* in the broiler supply chain in The Netherlands. *Journal of Food Protection* 2008; **71**: 1974–1980.
17. **Heyndrickx M, *et al.*** Routes for *salmonella* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. *Epidemiology and Infection* 2002; **129**: 253–265.
18. **Lai J, *et al.*** Serotype distribution and antibiotic resistance of *Salmonella* in food-producing animals in Shandong province of China, 2009 and 2012. *International Journal of Food Microbiology* 2014; **180**: 30–38.
19. **Berghaus RD, *et al.*** Multilevel analysis of environmental *Salmonella* prevalences and management practices on 49 broiler breeder farms in four southeastern States, USA. *Zoonoses Public Health* 2012; **59**: 365–374.
20. **Lu Y, *et al.*** Prevalence of antimicrobial resistance among *Salmonella* isolates from chicken in China. *Foodborne Pathogens and Disease* 2011; **8**: 45–53.
21. **Schwaiger K, *et al.*** Prevalence of antibiotic-resistant Enterobacteriaceae isolated from chicken and pork meat purchased at the slaughterhouse and at retail in Bavaria, Germany. *International Journal of Food Microbiology* 2012; **154**: 206–211.
22. **van Asselt ED, *et al.*** *Salmonella* serotype distribution in the Dutch broiler supply chain. *Poultry Science* 2009; **88**: 2695–2701.
23. **Li R, *et al.*** Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China. *International Journal of Food Microbiology* 2013; **163**: 14–18.
24. **Zhu J, *et al.*** Prevalence and quantification of *Salmonella* contamination in raw chicken carcasses at the retail in China. Food Control 2014; **44**: 198–202.
25. **Marin C, *et al.*** Sources of *Salmonella* contamination during broiler production in Eastern Spain. *Preventive Veterinary Medicine* 2011; **98**: 39–45.
26. **Choi SW, *et al.*** Prevalence and characterization of *Salmonella* species in entire steps of a single integrated broiler supply chain in Korea. *Poultry Science* 2014; **93**: 1251–1257.
27. **Nakao JH, *et al.*** ‘One Health’ investigation: outbreak of human *Salmonella* Braenderup infections traced to a mail-order hatchery – United States, 2012–2013. *Epidemiology and Infection* 2015; **143**: 2178–2186.
28. **Heyndrickx M, *et al.*** Routes for *Salmonella* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. *Epidemiology and Infection* 2002; **129**: 253–265.
29. **Gong J, *et al.*** Prevalence and fimbrial genotype distribution of poultry *Salmonella* isolates in China (2006 to 2012). *Applied Environmental Microbiology* 2014; **80**: 687–693.
30. **Liang Z, *et al.*** Serotypes, seasonal trends, and antibiotic resistance of non-typhoidal *Salmonella* from human patients in Guangdong Province, China, 2009–2012. *BMC Infectious Diseases*. 2015; **15**: 53.
31. **Bangtrakulnonth A, *et al.*** *Salmonella* serovars from humans and other sources in Thailand, 1993–2002. *Emerging Infectious Diseases* 2004; **10**: 131–136.
32. **Utrarachkij F, *et al.*** Possible horizontal transmission of *Salmonella* via reusable egg trays in Thailand. *International Journal of Food Microbiology* 2012; **154**: 73–78.
33. **Ponce E, *et al.*** Prevalence and characterization of *Salmonella enterica* serovar Weltevreden from imported seafood. *Food Microbiology* 2008; **25**: 29–35.
34. **Brankatschk K, *et al.*** Genome of a European fresh-vegetable food safety outbreak strain of *Salmonella enterica* subsp. *enterica* serovar Weltevreden. *Journal of Bacteriology* 2011; **193**: 2066.
35. **Alali WQ, *et al.*** Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. *Foodborne Pathogens and Disease* 2010; **7**: 1363–1371.
36. **Poros-Gluchowska J, Markiewicz Z.** Antimicrobial resistance of *Listeria* monocytogenes. *Acta Microbiologica Polonica* 2003; **52**: 113–129.
37. **Barbosa TM, Levy SB.** The impact of antibiotic use on resistance development and persistence. *Drug Resistance Updates* 2000; **3**: 303–311.
38. **Diarra MS, *et al.*** Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and enterococcus counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates. *Applied and Environmental Microbiology* 2007; **73**: 6566–6576.
39. **Lu Y, *et al.*** Prevalence of antimicrobial resistance among *Salmonella* isolates from chicken in China. *Foodborne Pathogens and Disease* 2011; **8**: 45–53.
40. **Zhang J, *et al.*** Laboratory monitoring of bacterial gastroenteric pathogens *Salmonella* and *Shigella* in Shanghai, China 2006–2012. *Epidemiology and Infection*. 2015; **143**: 478–485.

https://doi.org/10.1017/S0950268816001515 Published online by Cambridge University Press

41. **Osman KM, Hassan WM, Mohamed RA.** The consequences of a sudden demographic change on the seroprevalence pattern, virulence genes, identification and characterisation of integron-mediated antibiotic resistance in the *Salmonella enterica* isolated from clinically diarrhoeic humans in Egypt. *European Journal of Clinical Microbiology Infectious Diseases* 2014; **33**: 1323–1337.
42. **Hapfelmeier S, *et al*.** Role of the *Salmonella* pathogenicity island 1 effector proteins SipA, SopB, SopE, and SopE2 in *Salmonella enterica* subspecies 1 serovar Typhimurium colitis in streptomycin-pretreated mice. *Infection and Immunity* 2004; **72**: 795–809.
43. **Aslanzadeh J, Paulissen LJ.** Adherence and pathogenesis of *Salmonella enteritidis* in mice. *Microbiology and Immunology* 1990; **34**: 885–893.
44. **Guiney DG, *et al*.** Plasmid-mediated virulence genes in non-typhoid *Salmonella* serovars. *FEMS Microbiology Letters* 1994; **124**: 1–9.
45. **Lynne AM, *et al*.** Characterization of antimicrobial resistance in *Salmonella enterica* serotype Heidelberg isolated from food animals. *Foodborne Pathogens and Disease* 2009; **6**: 207–215.
46. **Prager R, *et al*.** Prevalence and polymorphism of genes encoding translocated effector proteins among clinical isolates of *Salmonella enterica*. *International Journal of Medical Microbiology* 2000; **290**: 605–617.
47. **Soto SM, *et al*.** Detection of virulence determinants in clinical strains of *Salmonella enterica* serovar Enteritidis and mapping on macrorestriction profiles. *Journal of Medical Microbiology* 2006; **55**: 365–373.
48. **Mikasova E, *et al*.** Characterization of *Salmonella enterica* serovar Typhimurium strains of veterinary origin by molecular typing methods. *Veterinary Microbiology* 2005; **109**: 113–120.
49. **Herrero A, *et al*.** Molecular epidemiology of emergent multidrug-resistant *Salmonella enterica* serotype Typhimurium strains carrying the virulence resistance plasmid pUO-StVR2. *Journal of Antimicrobial Chemotherapy* 2006; **57**: 39–45.
50. **Chiu CH, Ou JT.** Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture-multiplex PCR combination assay. *Journal of Clinical Microbiology* 1996; **34**: 2619–2622.
51. **Huehn S, *et al*.** Virulotyping and antimicrobial resistance typing of *Salmonella enterica* serovars relevant to human health in Europe. *Foodborne Pathogens and Disease* 2010; **7**: 523–535.

https://doi.org/10.1017/S0950268816001515 Published online by Cambridge University Press