Accepted Manuscript

Prevalence, sequence types, antibiotic resistance and, *gyrA* mutations of *Salmonella* isolated from retail fresh chicken meat in Singapore

Ye Htut Zwe, Vivien Chia Yen Tang, Kyaw Thu Aung, Ramona Alikiiteaga Gutiérrez, Lee Ching Ng, Hyun-Gyun Yuk

PII: S0956-7135(18)30101-4

DOI: 10.1016/j.foodcont.2018.03.004

Reference: JFCO 6018

To appear in: *Food Control*

Received Date: 5 January 2018

Revised Date: 27 February 2018

Accepted Date: 1 March 2018

Please cite this article as: Zwe Y.H., Yen Tang V.C., Aung K.T., Gutiérrez R.A., Ng L.C. & Yuk H.-G., Prevalence, sequence types, antibiotic resistance and, *gyrA* mutations of *Salmonella* isolated from retail fresh chicken meat in Singapore, *Food Control* (2018), doi: 10.1016/j.foodcont.2018.03.004.

Prevalence, sequence types, antibiotic resistance and, *gyrA* mutations of *Salmonella* isolated from retail fresh chicken meat in Singapore

Ye Htut Zwe [a], Vivien Chia Yen Tang [a], Kyaw Thu Aung [b,c], Ramona Alikiiteaga Gutiérrez [b], Lee Ching Ng [b,d], Hyun-Gyun Yuk [e,*]

[a] Food Science and Technology Programme, Department of Chemistry, National University of Singapore, Science Drive 4, Singapore 117543

[b] Environmental Health Institute, National Environment Agency, 11 Biopolis Way, Helios Block #06-05/08 Singapore 138667

[c] School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive Singapore 637459

[d] School of Biological Science, Nanyang Technological University, 60 Nanyang Drive Singapore 637551

[e] Department of Food Science and Technology, Korea National University of Transportation, 61 Daehak-ro, Jeungpyeong-gun, Chungbuk, Republic of Korea 27909

* Corresponding author. Department of Food Science and Technology, Korea National University of Transportation, 61 Daehak-ro Jeungpyeong-gun, Chungbuk, Republic of Korea 27909

Tel: +82-43-820-5244; *E-mail address*: yukhg@ut.ac.kr (H.G. Yuk)

ABSTRACT

Salmonellosis, caused by multidrug-resistant *Salmonella* species, in particular, is one of the leading notifiable foodborne diseases in Singapore and an emerging public health concern worldwide. The objectives of this study were to determine the prevalence, antibiotic resistance and the presence of *gyrA* and *parC* mutations in *Salmonella* isolated from retail fresh chicken meat in Singapore. A significantly higher prevalence of *Salmonella* was found in chicken meat from the wet markets (25%, 30/120), as compared to supermarkets (12.7%, 19/150). The top four serovars isolated in this study were *S.* Saintpaul (32.7%, 17/52), followed by *S.* Brancaster (21.2%, 11/52), *S.* Albany (11.5%, 6/52), and *S.* Stanley (9.6%, 5/52). More than 80% of *Salmonella* isolates exhibited resistance to at least one of eleven antibiotics tested. The most common phenotypic resistances exhibited were towards ampicillin (78.8%, 41/52), tetracycline and chloramphenicol (61.5%, 32/52), sulfamethoxazole-trimethoprim (55.8%, 29/52) and nalidixic acid (30.8%, 16/52). Of the 52 *Salmonella* isolates, 59.6% (31/52) were multi-drug resistant strains, resistant to 3 or more antibiotic classes. No mutation in *parC* gene was found in any of the isolates that expressed phenotypic resistance or reduced susceptibility towards quinolone and/or fluoroquinolone. Mutations at two different sites of *gyrA* gene were found in 7 isolates, which further discriminates the quinolone resistance genotype in *Salmonella* isolates from fresh chicken meat. Our findings provide opportunities for risk assessment and management of salmonellosis and antibiotic-resistant *Salmonella* species in the country and the region.

47 *Keywords: Salmonella*, chicken, sequence types, antibiotic resistance, quinolone

48 resistance

1. Introduction

Non-typhoidal salmonellosis caused by *Salmonella* is one of the most common foodborne diseases worldwide. In the United States, salmonellosis has been estimated to be responsible for 1 million illnesses and attributed to 400 deaths each year (Scallan, et al., 2011), and up to 155,000 deaths globally (Majowicz, et al., 2010). In Singapore, non-typhoidal salmonellosis contributed 56% of all notifiable foodborne and waterborne diseases from 2001 to 2010 (Kondakci & Yuk, 2012). The incidence (per 100,000 population) of non-typhoidal salmonellosis in Singapore has been rising from 26.5 in 2011 to 35.9 in 2015 due, in part, to the mandatory notification requirement of salmonellosis implemented in 2008 (MOH, 2012, 2016; Zwe & Yuk, 2017).

Chicken meat is one of the most commonly implicated food for human salmonellosis (Vandeplas, Dauphin, Beckers, Thonart, & Thewis, 2010). Owing to its ability to reside in healthy chickens without causing illness (de Jong, et al., 2014), *Salmonella* may go unnoticed in the farm, thereby posing the risk of salmonellosis through contaminated chickens.

The administration of antibiotics in livestock as growth promoters has been a long-standing agricultural practice and is primarily associated with the emergence of antibiotic resistance in foodborne and livestock-associated bacteria (Thames, Pruden, James, Ray, & Knowlton, 2012). In particular, multidrug-resistant (MDR) *Salmonella* has been consistently reported in food-producing animals and food globally in the recent decade (Abd-Elghany, Sallam, Abd-Elkhalek, & Tamura, 2015; Anjum, et al., 2011; Lettini, et al., 2016; Nguyen, et al., 2016; Oueslati, et al., 2016; Rodriguez-Rivera, et al., 2016),

resulting in humans being exposed to MDR *Salmonella* through food. Although mostly causing self-limiting gastroenteritis, *Salmonella* infections can be invasive and potentially fatal, especially in children, elderly and immunocompromised individuals (Arshad, et al., 2008). Administration of antibiotics becomes essential in these cases, with the drugs of choice being fluoroquinolones in most cases (Kariuki, Gordon, Feasey, & Parry, 2015). Hence, reports indicating evidence of increasing resistance to fluoroquinolones in *Salmonella* (Lettini, et al., 2016; Veldman, et al., 2011; Zhang, et al., 2014) are of concern because it could mean a limited choice of therapeutics in the near future.

In Singapore, the Ministry of Health (MOH) annually reports surveillance data of reported cases of human salmonellosis. However, to our knowledge, there is no report on the contamination rates and antibiotic resistance of *Salmonella* in raw meat in Singapore. Thus, the objectives of this study were to determine the prevalence, sequence types/serovars and antibiotic resistance patterns of *Salmonella* from fresh chicken meat available in wet markets and supermarkets in Singapore. Furthermore, possible mechanisms of fluoroquinolone and quinolone resistance in *Salmonella* isolated from chicken meat were investigated. Findings from this study may help authorities in assessing the possible exposure risk of *Salmonella* from contaminated chicken meat for developing risk mitigation measures, and in evaluating the risk of antibiotic resistance hazard in the food supply chain in Singapore and elsewhere. In addition, they also serve as valuable data points in future long-term surveillance programs of *Salmonella* contamination patterns and/or antibiotic resistance patterns in the region.

2. Materials and methods

95

96 *2.1. Sample collection and preparation*

97

98 Sample collection was done between June 2015 and April 2016. Two types of
99 markets, namely wet markets and supermarkets, were included in this study for sample
100 collection. A total of 120 fresh (chilled) chicken samples were collected (30 breasts, 30
101 drum sticks, 30 thighs, 30 whole) from more than 30 different poultry vendors across 10
102 wet markets. A total of 150 fresh (chilled) chicken meat samples were collected (30
103 breasts, 30 drum sticks, 30 minced, 30 thighs, 30 whole) from 9 outlets across 3 different
104 supermarket chains. Each sample of approximately 100 g was individually wrapped
105 separately in new and clean plastic bags and transported on ice to the Food Microbiology
106 Laboratory of the National University of Singapore. All samples were either tested
107 immediately, or stored at 4 ^{o}C before being processed within 12 hours from the time of
108 collection.

109

110 *2.2. Isolation and serogrouping of* Salmonella *species*

111

112 Isolation of *Salmonella* from chicken meat samples was carried out according to the
113 ISO 6579:2002 (ISO, 2002) as performed previously (Zheng, Mikš-Krajnik, Yang, Xu, &
114 Yuk, 2014). Knives, steel trays and wooden chopsticks used to handle the chicken sample
115 during the portioning were all sterilized by autoclaving at 121 °C for 15 min in wrapped
116 aluminum foil prior to the experiment. The outer packaging of the samples was
117 decontaminated with 70% ethanol and left to dry in the biosafety cabinet (BSC; Esco

Class II, Type A2, E-Series, Esco Micro Pvt. Ltd., Singapore) before handling to prevent cross-contamination from the outer packaging to the sample. A 25 g portion of each sample was aseptically cut, portioned in the steel trays and weighed on a sterile Petri dish in the BSC. Bones were not included in the portions in this experiment. For whole chicken carcasses, the sample rinsate was obtained as recommended by the United States Department of Agriculture in the document MLG 4.08 (USDA, 2014). Briefly, the whole chicken carcass was transferred into a large sterile bag with a filter. A 400 ml of sterile BPW was poured into the cavity of the carcass to rinse inside out with a rocking motion for 1 min. A 30 ml portion of the sample rinsate was then transferred into a sterile bag containing 30 ml of sterile BPW and incubated at 37 °C for 24 h. Up to three presumptive *Salmonella* colonies from each of the xylose lysine deoxycholate agar (Oxoid) and brilliant green agar (Oxoid) were plated onto nutrient agar (Oxoid) to analyze their biochemical characteristics using API 20E (BioMérieux®, Inc., Marcy I'Etoile, France) that were interpreted by APIWEB software (BioMérieux®). Only colonies that displayed a ≥ 95% probability of being identified as *Salmonella* were taken for the study. If more than one *Salmonella* colony with identical biochemical fingerprints were isolated from a common sample, only one *Salmonella* colony per sample was chosen for further characterization. Serogrouping of the *Salmonella* isolates was carried out using the Wellcolex™ Colour Salmonella Rapid Latex Agglutination Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions.

2.3. Multilocus sequence typing (MLST) of Salmonella *species*

Genomic DNA was extracted from the isolates using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific). MLST was performed by amplifying and sequencing the seven housekeeping gene fragments with primers as described in Table 1. Amplifications were carried out in 50 µl reaction volume containing 10 µl of 5x Phusion HF buffer (Thermo Fisher Scientific), 1 µl of 10 mM dNTP mix (1st BASE, Selangor, Malaysia), 1 µl each of 10 µM forward and reverse primers and 0.5 µl of Phusion Hot Start II Taq DNA polymerase (Thermo Fisher Scientific) topped up with 31.5 µl nuclease-free water (Ambion®, Thermo Fisher Scientific). The PCR was performed as follows: 98 °C for 30 sec followed by 35 cycles of denaturation (98 °C for 10 sec), annealing (55 °C for 30 sec) and extension (72 °C for 30 sec). A final extension step was carried out at 72 °C for 10 min using a Veriti 96 Well Thermal Cycler (Applied Biosystems, Carlsbad, CA, USA). The PCR products were visualized by gel electrophoresis in 2% agarose (1st BASE).

PCR amplicons of the MLST genes were purified using the GeneAll Expin™ Gel SV kit (GeneAll Biotechnology, Seoul, Korea) and sequenced with sequencing primers as shown in Table 1 using capillary electrophoresis with Applied Biosystems® 3730/3730xl DNA Analyzer and BigDye Terminator v3.1 (Thermo Fisher Scientific). The reference templates for the seven MLST genes were obtained from the University of Warwick (UoW) MLST database (Warwick). Sequences of the PCR products were then assembled using the SeqMan Pro version 8.0 (DNASTAR). The obtained consensus sequence of each gene was then entered into the UoW MLST database to obtain the corresponding allele number for each gene sequence. Sequence type (ST) of each *Salmonella* isolate was obtained by entering in the combination of allele types of the

seven genes into the UoW MLST database. The corresponding serovar to the obtained ST was then ascertained by referring to the database (Achtman, et al., 2012). Serovars of ST3633 isolates that could not be ascertained with confidence from the database alone were serotyped by slide agglutination test using *Salmonella* antisera (Bio-Rad Laboratories, Hercules, CA, USA).

A dendrogram displaying the phylogenetic relationships between different *Salmonella* isolates was constructed using Molecular Evolutionary Genetics Analysis (MEGA) version 7.0, using the Neighbour-Joining (NJ) method based on concatenated 7 house-keeping genes determined by MLST.

2.4. Antibiotic susceptibility testing (AST) of Salmonella *species*

The antibiotic susceptibilities of the *Salmonella* isolates to 11 types across 7 classes of antibiotics were tested using the disk diffusion method and the results were interpreted according to the breakpoints described by the Clinical and Laboratory Standards Institute (CLSI, 2014). The antibiotic disks (Oxoid) used in the study were as follows: 10 µg ampicillin (AMP), 20 µg amoxicillin and 10 µg clavulanate (AMC), 30 µg ceftriaxone (CRO), 30 µg tetracycline (TE), 5 µg ciprofloxacin (CIP), 10 µg norfloxacin (NOR), 30 µg nalidixic acid (NAL), 23.75 µg sulfamethoxazole and 1.25 µg trimethoprim (SXT), 30 µg chloramphenicol (C), 30 µg amikacin (AMK) and 10 µg gentamicin (CN). The isolates were classified as either susceptible, intermediate or resistant as per the CLSI breakpoints. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as quality control organisms in the AST. A multidrug-resistant (MDR) isolate

was defined as an isolate exhibiting resistance towards three or more different antibiotic classes as previously described (Nguyen, et al., 2016).

2.5. Detection of mutations in gyrA *and* parC *genes*

The genetic features associated with resistance of the isolates to quinolone and fluoroquinolone were assessed as previously described by Šeputienė, et al. (2006). The 32 isolates that displayed either resistant or intermediate phenotype towards nalidixic acid and/or ciprofloxacin was analyzed for the presence of any mutations in *gyrA* and *parC* genes. The quinolone resistance determining region (QRDR) of *gyrA* and *parC* were amplified using the primers (Table 1) in reaction volumes as per the MLST methodology described in this study. The PCR protocol used for the amplification was as described by Šeputienė, et al. (2006). The PCR products were purified and sequenced as described previously. The reference templates of *gyrA* and *parC* were downloaded from GenBank at the National Centre for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov) from a *S.* Saintpaul whole genome with accession number GCA_000170215.1. The PCR product sequences were then aligned to the reference gene templates, trimmed and assembled as described previously and converted into protein sequences using the online translation tool publicly available at ExPASy (http://web.expasy.org/translate). The mutations in the QRDR of *gyrA* and *parC* amino acid sequences leading to quinolone and fluoroquinolone resistance were assessed by referring to a panel of known mutations previously compiled and published in supplementary materials by Stoesser, et al. (2013).

2.6. Statistical analysis

Statistical comparison of the prevalence of *Salmonella* by market type and parts was carried out using Fisher's exact test available online at the GraphPad Software (https://graphpad.com/quickcalcs/contingency1.cfm) with a significance level set at $P < 0.05$. The 95% confidence intervals were calculated using the Adjusted Wald method available online at MeasuringU (https://measuringu.com/wald/).

3. Results

3.1. Prevalence of Salmonella *species in chicken meat from wet markets and supermarkets*

Of the 270 chicken meat samples collected from wet markets and supermarkets, 52 *Salmonella* isolates were obtained from 49 samples, resulting in a prevalence of 18.1% (Table 2). Of the 49 samples detected with *Salmonella*, 30 samples were from wet markets, while 19 samples were from supermarkets, resulting in a prevalence of 25.0% (30/120) and 12.7% (19/150), respectively. The prevalence of *Salmonella* in chicken meat from wet markets was significantly higher ($P < 0.05$) than that from supermarkets. No significant difference ($P \geq 0.05$) in the prevalence between different chicken parts was observed.

A total of 11 different serovars were detected among the 52 *Salmonella* isolates, with the four most frequently isolated serovars being *S.* Saintpaul (32.7%, 17/52), followed by *S.* Brancaster (21.2%, 11/52), *S.* Albany (11.5%, 6/52), and *S.* Stanley (9.6%, 5/52) (Table 3). A wider variety of serovars was isolated from the chicken meat samples collected from wet markets (10 serovars) as compared to the samples from supermarkets (3 serovars). *S.* Saintpaul and *S.* Brancaster were detected in both market types and 70% (14/20) of all *Salmonella* isolates from supermarkets' samples were *S.* Saintpaul as opposed to 9.4% (3/32) of isolates from wet markets' samples. Isolates of ST3633 were found to be *S.* Albany from the slide agglutination test.

3.2. Distribution of sequence types and serovars based on MLST

A total of 12 distinct sequence types (STs) across 11 serovars were isolated in this study (Fig. 1). Two distinct STs (292 and 3633) were found among the *S.* Albany isolates.

3.3. Antibiotic resistance of Salmonella *in fresh chicken meat*

Antibiotic susceptibility testing (AST) revealed that 80.8% (42/52) of isolates were resistant to at least one of the antibiotics tested in this study. The most common phenotypic resistance exhibited was towards ampicillin followed by tetracycline, chloramphenicol, sulfamethoxazole-trimethoprim and nalidixic acid (Fig. 2). Relatively low resistance rates (< 10%) were found for ceftriaxone and ciprofloxacin while no

resistance towards amikacin and norfloxacin was found. Of the 52 isolates, 59.6% (31/52) of them were multidrug resistant (MDR) isolates (Table 4). The percentage of MDR isolates resistant to 3, 4, 5 and 6 classes of antibiotics were 1.9, 17.3, 28.8 and 11.5% respectively. All *S.* Albany isolates (n=6) were found to be resistant to at least five different classes of antibiotics while all *S.* Agona isolates (n=4) were susceptible to all antibiotics tested. One isolate of *S.* Albany was found to be resistant to 8 antibiotics with a resistance phenotype of AMC-AMP-C-CRO-CIP-NA-SXT-TE. The most common resistance phenotype among MDR isolates was AMP-C-SXT-TE (7 isolates, 13.5%) followed by AMP-C-CN-SXT-TE (6 isolates, 11.5%) (Table 4).

3.4. Investigation of genetic resistance determinants to quinolones

No mutation in the QRDR of *parC* gene was observed in any of the isolates tested (Table 5). A *gyrA* mutation responsible for changing the aspartic acid to asparagine residue at the 87th position (Asp87Asn) was found in *S.* Albany isolates (n=6) while a mutation causing a change in serine to tyrosine residue at the 83rd position (Ser83Tyr) was found in *S.* Give isolate (n=1). *S.* Brancaster and *S.* Stanley isolates which displayed a resistant phenotype to nalidixic acid did not display any mutations in either *gyrA* or *parC* genes. One isolate (WB2) among *S.* Albany isolates carrying the Asp87Asn mutation, and the *S.* Give isolate carrying the Ser83Tyr mutation, displayed phenotypic resistance towards both nalidixic acid and ciprofloxacin.

4. Discussion

278

279 To our knowledge, there is no other comparable published study on *Salmonella*
280 contamination rates of raw meat items in Singapore. Hence, the prevalence rate found in
281 this study could not be compared to any local past data to establish any trend.
282 Internationally, this *Salmonella* prevalence rate of 18.1% in raw chicken in Singapore is
283 lower compared to values reported in Vietnam (21.0 % to 65.3% in poultry; Huong, et al.,
284 2006; Nguyen, et al., 2016; Phan, et al., 2005; Ta, et al., 2014; Van, Moutafis, Istivan,
285 Tran, & Coloe, 2007), Egypt (34% in chicken; Abd-Elghany, et al., 2015), Louisiana,
286 United States (21.7% in chicken; Lestari, Han, Wang, & Ge, 2009), Maryland, United
287 States (56% in chicken; Cui, Ge, Zheng, & Meng, 2005), Anatolia (34% in chicken;
288 Yildirim, Gonulalan, Pamuk, & Ertas, 2011), Mexico (35.3% in poultry; Miranda,
289 Mondragon, Martinez, Guarddon, & Rodriguez, 2009) and Penang, Malaysia (23.5% in
290 ducks; Adzitey, Rusul, & Huda, 2012). The prevalence found in this study is higher
291 compared to those in New Zealand (3% in chicken; Wong, Nicol, Cook, & MacDiarmid,
292 2007) and United Kingdom (4% in chicken; Meldrum & Wilson, 2007). However, in
293 these countries, *Campylobacter* species tend to be the main contaminant of raw chicken
294 instead (Baker, et al., 2006; Meldrum & Wilson, 2007). The lower *Salmonella* prevalence
295 rate in Singapore as compared to many countries could be attributed to the governance of
296 food safety and hygiene in Singapore by the combined efforts of the Agri-Food &
297 Veterinary Authority of Singapore (AVA) and the National Environment Agency (NEA)
298 as succinctly documented by Ludher (2015).

299 Our study showed that the prevalence of *Salmonella* contamination in raw chicken
300 from wet markets was significantly higher than in those from the supermarkets. These

301 results suggest that the prevalence of *Salmonella* species may vary depending on the type
302 of retail establishments. Relatively inferior hygiene practices, such as cutting chicken
303 meat with shared knife and chopping board without proper cleaning, and displaying
304 chicken carcasses in the chillers without physical separation or individual packaging
305 could likely contribute to cross-contamination events leading to a significantly higher rate
306 of *Salmonella* contamination in fresh chicken meat sold in wet markets.

307 We found that *S.* Saintpaul, *S.* Brancaster, *S.* Albany, and *S.* Stanley represented up to
308 75% of all the isolates in fresh chicken meat from wet markets and supermarkets. The
309 most prevalent serovar from raw chicken vary widely across studies: *S.* Corvallis and *S.*
310 Albany in Vietnam (Nguyen, et al., 2016; Ta, et al., 2014) and *S.* Enteritidis in Egypt and
311 China (Abd-Elghany, et al., 2015; Li, Zhou, & Miao, 2017), suggesting geography-
312 dependent prevalence pattern of serovars. Based on Singapore's Ministry of Health's
313 epidemiological reports, the top four serovars in this study are attributed to approximately
314 10% of human salmonellosis cases in Singapore (MOH, 2015). On the other hand,
315 *Salmonella* Enteritidis, the most commonly reported serovar responsible for human
316 salmonellosis cases in the last decade in Singapore (Kondakci & Yuk, 2012), was not
317 detected in our cross-sectional study. This suggests that fresh chicken meat may not play
318 a major role in the epidemiology of salmonellosis due to *S.* Enteritidis in Singapore.
319 Further studies involving other food items such as eggs may be needed to explain the
320 observation. Alternatively, due to the detection limit of the culture method (1 CFU/25 g
321 or 0.04 CFU/g), any *S.* Enteritidis cells present in the fresh raw chicken below the
322 detection method may not be isolated but still might cause disease due to its higher
323 virulence (Teunis, et al., 2010).

In this study, we successfully used multilocus sequence typing (MLST) as an alternative method to the conventional serotyping scheme of *Salmonella* isolates as suggested by (Achtman, et al., 2012). This addressed the high cost needed to maintain a large collection of 'O' and 'H' *Salmonella* antigens required in serotyping a wide array of common and rare serovars alike. Although effective as a serotyping tool, the determination of phylogeny using MLST may have some limitations in discriminating power, due to the relatively low variable nature of the seven housekeeping genes. In our study, apart from *S.* Albany which was isolated in the form of two different sequence types (STs), for all other isolates, only one ST each was found for each serovar. In the dendrograms based on MLST, isolates with identical STs are clustered together and no further genotypic discrimination is possible even though they may be isolated from different sources, market types and time period. An alternative genotyping method with a higher resolution, such as whole genome sequencing (WGS) followed by the construction of phylogenetic single nucleotide polymorphism (SNP) tree may help to determine the genetic relationship between isolates of the same serovar from different sources as previously demonstrated by Leekitcharoenphon, Nielsen, Kaas, Lund, and Aarestrup (2014) and Holt, et al. (2010). Nevertheless, isolates displaying different STs such as *S.* Albany (ST292 and ST3633) in this study appeared to be genetically different and hence can be discriminated based on MLST.

We detected multidrug-resistant (MDR) *Salmonella* in 59.6% (31/52) of the isolates obtained from fresh chicken meat sold in local wet markets and supermarkets. The most common phenotypic resistance pattern among MDR isolates was AMP-C-SXT-TE. Previous studies reported similar phenotypes in *Salmonella* isolates from food-producing

347 animals in Vietnam (Lettini, et al., 2016; Nguyen, et al., 2016; Ta, et al., 2014; Thai,
348 Hirai, Lan, & Yamaguchi, 2012; Thai & Yamaguchi, 2012; Tu, et al., 2015), Mexico
349 (Miranda, et al., 2009) and Thailand (Pulsrikarn, et al., 2012). The use of penicillin,
350 sulfonamides and tetracycline as primary treatment of various bacterial diseases in
351 poultry (Mathew, Cissell, & Liamthong, 2007; Singer & Hofacre, 2006) might have
352 contributed to the emergence of this phenotype as the dominant resistance pattern.
353 Considering the high rates of resistance towards these antibiotics found in this study and
354 several others, strict regulation and extensive education in the use of these drugs in
355 agriculture as well as in human and veterinary medicine should be implemented. This
356 will serve to prevent further emergence of resistance that could seriously limit their
357 usefulness in the near future.

358 To understand the genetic basis of resistance towards quinolone and fluoroquinolone
359 which are of clinical importance in the treatment of invasive salmonellosis (Acheson &
360 Hohmann, 2001), the presence of mutations of *gyrA* and *parC* in the QRDR were
361 assessed. There are several known mechanisms of resistance towards quinolones and
362 fluoroquinolones: target gene mutations, active efflux pumps, a decreased outer
363 membrane permeability and plasmid-mediated resistance genes (Hooper, 2001; Jacoby,
364 2005; Ruiz, 2003; Zgurskaya & Nikaido, 2000). In this study, we investigated the
365 stepwise mutations between alternating gene targets *gyrA* and *parC* (Hooper, 2001). We
366 found that *S*. Give (WB11) and *S*. Albany (WB2) isolates carried a single mutation in the
367 primary target (*gyrA*) but not in the secondary target (*parC*) despite exhibiting phenotypic
368 resistance to both nalidixic acid and ciprofloxacin (Table 5). This was not in agreement
369 with the findings of Šeputienė, et al. (2006) who reported that a first mutation in *gyrA*

gene conferred only nalidixic acid resistance while a second mutation in *parC* was needed before developing ciprofloxacin resistance in Gram-negative bacteria. Unexpected ciprofloxacin resistance in *S.* Give (WB11) and *S.* Albany (WB2) isolates in this study in addition to the predicted resistance to nalidixic acid suggests the presence of other resistance mechanisms synergistically at work with the *gyrA* mutation. Furthermore, nalidixic acid resistance displayed by *S.* Stanley and *S.* Brancaster isolates despite lack of any mutations in *gyrA* and *parC* genes also suggests the presence of other resistance mechanisms such as the plasmid-borne *qnr* resistance genes. An investigation of the presence of *qnr* genes should be carried out in these isolates next to further establish a complete picture of quinolone resistance in these isolates.

5. Conclusion

This is the first report from Singapore that determines the prevalence and antibiotic resistance patterns of *Salmonella* isolated from retail fresh chicken meat. Significantly higher prevalence of *Salmonella* contamination was found in chicken meat from the wet markets than those from supermarkets, suggesting that the degree of *Salmonella* contamination might be market type-dependent. Of the 52 *Salmonella* isolates, 59.6% were multidrug resistant that exhibited high resistance rates towards ampicillin, chloramphenicol, tetracycline and sulfamethoxazole-trimethoprim. Two different mutations in *gyrA*, but no *parC* mutation, were found in quinolone-resistant *Salmonella* isolates. The data provide opportunities for risk assessment and management of antibiotic-resistant *Salmonella* in the country and the region.

Acknowledgements

This study was supported in part by the National University of Singapore (NUS), and by the National Environment Agency (NEA), Singapore. The authors wish to thank Professor Teo Yik Ying from the Saw Swee Hock School of Public Health, NUS for guidance on the statistical analysis. No conflict of interest declared.

References

Abd-Elghany, S. M., Sallam, K. I., Abd-Elkhalek, A., & Tamura, T. (2015). Occurrence, genetic characterization and antimicrobial resistance of *Salmonella* isolated from chicken meat and giblets. *Epidemiology and Infection, 143*(05), 997-1003.

Acheson, D., & Hohmann, E. L. (2001). Nontyphoidal salmonellosis. *Clinical Infectious Diseases, 32*(2), 263-269.

Achtman, M., Wain, J., Weill, F.X., Nair, S., Zhou, Z., Sangal, V., Krauland, M. G., Hale, J. L., Harbottle, H., & Uesbeck, A. (2012). Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathog, 8*(6), e1002776.

Adzitey, F., Rusul, G., & Huda, N. (2012). Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang, Malaysia. *Food Research International, 45*(2), 947-952.

Anjum, M. F., Choudhary, S., Morrison, V., Snow, L. C., Mafura, M., Slickers, P., Ehricht, R., & Woodward, M. J. (2011). Identifying antimicrobial resistance

genes of human clinical relevance within *Salmonella* isolated from food animals in Great Britain. *Journal of Antimicrobial Chemotherapy, 66*(3), 550-559.

Arshad, M. M., Wilkins, M. J., Downes, F. P., Rahbar, M. H., Erskine, R. J., Boulton, M. L., Younus, M., & Saeed, A. M. (2008). Epidemiologic attributes of invasive non-typhoidal *Salmonella* infections in Michigan, 1995–2001. *International Journal of Infectious Diseases, 12*(2), 176-182.

Baker, M., Wilson, N., Ikram, R., Chambers, S., Shoemack, P., & Cook, G. (2006). Regulation of chicken contamination is urgently needed to control New Zealand's serious campylobacteriosis epidemic. *The New Zealand Medical Journal (Online), 119*(1243), U2264.

CLSI. (2014). Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI document M100-S24.

Cui, S., Ge, B., Zheng, J., & Meng, J. (2005). Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. *Applied and Environmental Microbiology, 71*(7), 4108-4111.

de Jong, A., Smet, A., Ludwig, C., Stephan, B., De Graef, E., Vanrobaeys, M., & Haesebrouck, F. (2014). Antimicrobial susceptibility of *Salmonella* isolates from healthy pigs and chickens (2008–2011). *Veterinary Microbiology, 171*(3-4), 298-306.

Holt, K. E., Baker, S., Dongol, S., Basnyat, B., Adhikari, N., Thorson, S., Pulickal, A. S., Song, Y., Parkhill, J., & Farrar, J. J. (2010). High-throughput bacterial SNP typing identifies distinct clusters of *Salmonella* Typhi causing typhoid in Nepalese children. *BMC Infectious Diseases, 10*(1), 144.

439 Hooper, D. C. (2001). Emerging mechanisms of fluoroquinolone resistance. *Emerging*
440 *Infectious Diseases, 7*(2), 337-341.
441 Huong, L. Q., Reinhard, F., Padungtod, P., Hanh, T. T., Kyule, M. N., Baumann, M. P.,
442 & Zessin, K. H. (2006). Prevalence of *Salmonella* in retail chicken meat in Hanoi,
443 Vietnam. *Annals of the New York Academy of Sciences, 1081*(1), 257-261.
444 ISO. (2002). International Organization for Standardization 6579:2002(E). Microbiology
445 of Food and Animal Feeding Stuffs — Horizontal Method for the Detection of
446 *Salmonella* spp.
447 Jacoby, G. A. (2005). Mechanisms of resistance to quinolones. *Clinical Infectious*
448 *Diseases, 41*(Supplement 2), S120-S126.
449 Kariuki, S., Gordon, M. A., Feasey, N., & Parry, C. M. (2015). Antimicrobial resistance
450 and management of invasive *Salmonella* disease. *Vaccine, 33*, C21-C29.
451 Kondakci, T., & Yuk, H. G. (2012). Overview of foodborne outbreaks in the last decade
452 in Singapore: alarming increase in nontyphoidal salmonellosis. *Food and*
453 *Beverage Asia. Pablo Publishing Pte Ltd., Singapore*, 42-45.
454 Leekitcharoenphon, P., Nielsen, E. M., Kaas, R. S., Lund, O., & Aarestrup, F. M. (2014).
455 Evaluation of whole genome sequencing for outbreak detection of *Salmonella*
456 *enterica*. *PloS one, 9*(2), e87991.
457 Lestari, S. I., Han, F., Wang, F., & Ge, B. (2009). Prevalence and antimicrobial resistance
458 of *Salmonella* serovars in conventional and organic chickens from Louisiana retail
459 stores. *Journal of Food Protection, 72*(6), 1165-1172.
460 Lettini, A. A., Vo Than, T., Marafin, E., Longo, A., Antonello, K., Zavagnin, P., Barco,
461 L., Mancin, M., Cibin, V., & Morini, M. (2016). Distribution of *Salmonella*

462 serovars and antimicrobial susceptibility from poultry and swine farms in central
463 Vietnam. *Zoonoses and Public Health, 63*(7), 569-576.
464 Li, S., Zhou, Y., & Miao, Z. (2017). Prevalence and antibiotic resistance of non-typhoidal
465 *Salmonella* isolated from raw chicken carcasses of commercial broilers and spent
466 hens in Tai'an, China. *Frontiers in Microbiology, 8*, 2106.
467 Ludher, E. (2015). A Case study of Singapore's Smart Governance of Food.
468 Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'brien, S. J., Jones, T.
469 F., Fazil, A., & Hoekstra, R. M. (2010). The global burden of nontyphoidal
470 *Salmonella* gastroenteritis. *Clinical Infectious Diseases, 50*(6), 882-889.
471 Mathew, A. G., Cissell, R., & Liamthong, S. (2007). Antibiotic resistance in bacteria
472 associated with food animals: a United States perspective of livestock production.
473 *Foodborne Pathogens and Disease, 4*(2), 115-133.
474 Meldrum, R. J., & Wilson, I. G. (2007). *Salmonella* and *Campylobacter* in United
475 Kingdom retail raw chicken in 2005. *Journal of Food Protection, 70*(8), 1937-
476 1939.
477 Miranda, J. M., Mondragon, A. C., Martinez, B., Guarddon, M., & Rodriguez, J. A.
478 (2009). Prevalence and antimicrobial resistance patterns of *Salmonella* from
479 different raw foods in Mexico. *Journal of Food Protection, 72*(5), 966-971.
480 MOH. (2012). Communicable Disease Surveillance in Singapore 2011.
481 MOH. (2015). Communicable Disease Surveillance in Singapore 2014.
482 MOH. (2016). Communicable Disease Surveillance in Singapore 2015.
483 Nguyen, D. T. A., Kanki, M., Nguyen, P. D., Le, H. T., Ngo, P. T., Tran, D. N. M., Le,
484 N. H., Dang, C. V., Kawai, T., Kawahara, R., Yonogi, S., Hirai, Y., Jinnai, M.,

485 Yamasaki, S., Kumeda, Y., & Yamamoto, Y. (2016). Prevalence, antibiotic
486 resistance, and extended-spectrum and AmpC β-lactamase productivity of
487 *Salmonella* isolates from raw meat and seafood samples in Ho Chi Minh City,
488 Vietnam. *International Journal of Food Microbiology, 236*, 115-122.
489 Oueslati, W., Rjeibi, M. R., Mhadhbi, M., Jbeli, M., Zrelli, S., & Ettriqui, A. (2016).
490 Prevalence, virulence and antibiotic susceptibility of *Salmonella* spp. strains,
491 isolated from beef in Greater Tunis (Tunisia). *Meat Science, 119*, 154-159.
492 Phan, T. T., Khai, L. T. L., Ogasawara, N., Tam, N. T., Okatani, A. T., Akiba, M., &
493 Hayashidani, H. (2005). Contamination of *Salmonella* in retail meats and shrimps
494 in the Mekong Delta, Vietnam. *Journal of Food Protection, 68*(5), 1077-1080.
495 Pulsrikarn, C., Chaichana, P., Pornruangwong, S., Morita, Y., Yamamoto, S., &
496 Boonmar, S. (2012). Serotype, antimicrobial susceptibility, and genotype of
497 *Salmonella* isolates from swine and pork in Sa Kaew province, Thailand. *The*
498 *Thai Journal of Veterinary Medicine, 42*(1), 21-28.
499 Rodriguez-Rivera, L. D., Cummings, K. J., Loneragan, G. H., Rankin, S. C., Hanson, D.
500 L., Leone, W. M., & Edrington, T. S. (2016). *Salmonella* prevalence and
501 antimicrobial susceptibility among dairy farm environmental samples collected in
502 Texas. *Foodborne Pathogens and Disease, 13*(4), 205-211.
503 Ruiz, J. (2003). Mechanisms of resistance to quinolones: target alterations, decreased
504 accumulation and DNA gyrase protection. *Journal of Antimicrobial*
505 *Chemotherapy, 51*(5), 1109-1117.

506 Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M.-A., Roy, S. L.,
507 Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United
508 States—major pathogens. *Emerging Infectious Diseases, 17*(1), 7-15.
509 Šeputienė, V., Povilonis, J., Ružauskas, M., Virgailis, M., Žlabys, P., & Sužiedėlienė, E.
510 (2006). Quinolone resistance among *Salmonella enterica* and *Escherichia coli* in
511 Lithuania. *Biologija*(3), 74-78.
512 Singer, R. S., & Hofacre, C. L. (2006). Potential impacts of antibiotic use in poultry
513 production. *Avian Diseases, 50*(2), 161-172.
514 Stoesser, N., Batty, E. M., Eyre, D. W., Morgan, M., Wyllie, D. H., Del Ojo Elias, C.,
515 Johnson, J. R., Walker, A. S., Peto, T. E. A., & Crook, D. W. (2013). Predicting
516 antimicrobial susceptibilities for *Escherichia coli* and *Klebsiella pneumoniae*
517 isolates using whole genomic sequence data. *Journal of Antimicrobial*
518 *Chemotherapy, 68*(10), 2234-2244.
519 Ta, Y. T., Nguyen, T. T., To, P. B., Pham, D. X., Le, H. T. H., Thi, G. N., Alali, W. Q.,
520 Walls, I., & Doyle, M. P. (2014). Quantification, serovars, and antibiotic
521 resistance of *Salmonella* isolated from retail raw chicken meat in Vietnam.
522 *Journal of Food Protection, 77*(1), 57-66.
523 Teunis, P. F. M., Kasuga, F., Fazil, A., Ogden, I. D., Rotariu, O., & Strachan, N. J. C.
524 (2010). Dose–response modeling of *Salmonella* using outbreak data. *International*
525 *Journal of Food Microbiology, 144*(2), 243-249.
526 Thai, T. H., Hirai, T., Lan, N. T., & Yamaguchi, R. (2012). Antibiotic resistance profiles
527 of *Salmonella* serovars isolated from retail pork and chicken meat in North
528 Vietnam. *International Journal of Food Microbiology, 156*(2), 147-151.

529 Thai, T. H., & Yamaguchi, R. (2012). Molecular characterization of antibiotic-resistant
530 *Salmonella* isolates from retail meat from markets in Northern Vietnam. *Journal*
531 *of Food Protection, 75*(9), 1709-1714.
532 Thames, C. H., Pruden, A., James, R. E., Ray, P. P., & Knowlton, K. F. (2012). Excretion
533 of antibiotic resistance genes by dairy calves fed milk replacers with varying
534 doses of antibiotics. *Frontiers in Microbiology, 3*, 139.
535 Tu, L. T. P., Hoang, N. V. M., Cuong, N. V., Campbell, J., Bryant, J. E., Hoa, N. T., Kiet,
536 B. T., Thompson, C., Duy, D. T., & Phat, V. V. (2015). High levels of
537 contamination and antimicrobial-resistant non-typhoidal *Salmonella* serovars on
538 pig and poultry farms in the Mekong Delta of Vietnam. *Epidemiology and*
539 *Infection, 143*(14), 3074-3086.
540 USDA. (2014). Isolation And Identification of *Salmonella* From Meat, Poultry,
541 Pasteurized Egg, and Catfish Products and Carcass and Environmental Sponges.
542 Van, T. T. H., Moutafis, G., Istivan, T., Tran, L. T., & Coloe, P. J. (2007). Detection of
543 *Salmonella* spp. in retail raw food samples from Vietnam and characterization of
544 their antibiotic resistance. *Applied and Environmental Microbiology, 73*(21),
545 6885-6890.
546 Vandeplas, S., Dauphin, R. D., Beckers, Y., Thonart, P., & Thewis, A. (2010).
547 *Salmonella* in chicken: current and developing strategies to reduce contamination
548 at farm level. *Journal of Food Protection, 73*(4), 774-785.
549 Veldman, K., Cavaco, L. M., Mevius, D., Battisti, A., Franco, A., Botteldoorn, N.,
550 Bruneau, M., Perrin-Guyomard, A., Cerny, T., & De Frutos Escobar, C. (2011).
551 International collaborative study on the occurrence of plasmid-mediated

quinolone resistance in *Salmonella enterica* and *Escherichia coli* isolated from animals, humans, food and the environment in 13 European countries. *Journal of Antimicrobial Chemotherapy, 66*(6), 1278-1286.

Warwick, U. o. MLST database at University of Warwick.

Wong, T. L., Nicol, C., Cook, R., & MacDiarmid, S. (2007). *Salmonella* in uncooked retail meats in New Zealand. *Journal of Food Protection, 70*(6), 1360-1365.

Yildirim, Y., Gonulalan, Z., Pamuk, S., & Ertas, N. (2011). Incidence and antibiotic resistance of *Salmonella* spp. on raw chicken carcasses. *Food Research International, 44*(3), 725-728.

Zgurskaya, H. I., & Nikaido, H. (2000). Multidrug resistance mechanisms: drug efflux across two membranes. *Molecular Microbiology, 37*(2), 219-225.

Zhang, Z., Meng, X., Wang, Y., Xia, X., Wang, X., Xi, M., Meng, J., Shi, X., Wang, D., & Yang, B. (2014). Presence of *qnr*, *aac (6')-Ib*, *qep A*, *oqx* AB, and mutations in gyrase and topoisomerase in nalidixic acid–resistant *Salmonella* isolates recovered from retail chicken carcasses. *Foodborne Pathogens and Disease, 11*(9), 698-705.

Zheng, Q., Mikš-Krajnik, M., Yang, Y., Xu, W., & Yuk, H. G. (2014). Real-time PCR method combined with immunomagnetic separation for detecting healthy and heat-injured *Salmonella* Typhimurium on raw duck wings. *International Journal of Food Microbiology, 186*, 6-13.

Zwe, Y. H., & Yuk, H. G. (2017). Food quality and safety in Singapore: microbiology aspects. *Food Quality and Safety, 1*(2), 101-105.

575

576 **Table 1.** Primers used in this study.

Gene Target	Primer	Sequence (5'-3')	Amplicon size (bp)	Reference
MLST amplification				(Warwick)
aroC	aroCF	CCTGGCACCTCGCGCTATAC	826	
	aroCR	CCACACACGGATCGTGGCG		
dnaN	dnaNF	ATGAAATTTACCGTTGAACGTGA	833	
	dnaNR	AATTTCTCATTCGAGAGGATTGC		
hemD	hemDF	ATGAGTATTCTGATCACCCG	666	
	hemDR	ATCAGCGACCTTAATATCTTGCCA		
hisD	hisDF	GAAACGTTCCATTCCGCGCAGAC	894	
	hisDR	CTGAACGGTCATCCGTTTCTG		
purE	purEF	ATGTCTTCCCGCAATAATCC	510	
	purER	TCATAGCGTCCCCCGCGGATC		
sucA	sucAF	AGCACCGAAGAGAAACGCTG	643	
	sucAR	GGTTGTTGATAACGATACGTAC		
thrA	thrAF	GTCACGGTGATCGATCCGGT	852	
	thrAR	CACGATATTGATATTAGCCCG		
MLST sequencing				(Warwick)
aroC	aroC_sF1	GGCGTGACGACCGGCAC		
	aroC_sR1	AGCGCCATATGCGCCAC		
dnaN	dnaN_sF	CCGATTCTCGGTAACCTGCT		
	dnaN_sR1	ACGCGACGGTAATCCGGG		
hemD	hemD_sF2	GCCTGGAGTTTTCCACTG		
	hemD_sR	GACCAATAGCCGACAGCGTAG		
hisD	hisD_sF	GTCGGTCTGTATATTCCCGG		

	hisD_sR	GGTAATCGCATCCACCAAATC		
purE	purE_sF1	ACAGGAGTTTTAAGACGCATG		
	purE_sR1	GCAAACTTGCTTCATAGCG		
sucA	sucA_sF1	CCGAAGAGAAACGCTGGATC		
	sucA_sR	GGTTGTTGATAACGATACGTAC		
thrA	thrA_sF	ATCCCGGCCGATCACATGAT		
	thrA_sR1	ACCGCCAGCGGCTCCAGCA		
Quinolone resistance-determining regions				(Šeputienė, et al., 2006)
gyrA	gyrAF	AAATCTGCCCGTGTCGTTGGT	343	
	gyrAR	GCCATACCTACTGCGATACC		
parC	parCF	GTGGTAGCGAAGAGGTGGTT	964	
	parCR	GACCGTGCG TTGCCGTTTAT		

577 Abbreviations: *aroC*, chorismate synthase; *dnaN*, DNA polymerase III beta subunit; *hemD*, uroporphyrinogen III cosynthase; *hisD*,

578 histidinol dehydrogenase; *purE*, phosphoribosylaminoimidazole carboxylase; *sucA*, alpha ketoglutarate dehydrogenase; *thrA*,

579 aspartokinase + homoserine dehydrogenase; *gyrA*, DNA gyrase subunit A; *parC*, topoisomerase subunit A.

580
581 **Table 2.** Prevalence[a] of *Salmonella* spp. isolated from chicken meat in Singapore.

Variable	No. of samples	No. (%) of *Salmonella*-positive sample	95% CI (%)
Overall	270	49 (18.1)	14.0 – 23.2
Market Type			
Wet market	120	30 (25.0) [A]	18.1 – 33.5
Supermarket	150	19 (12.7) [B]	8.2 – 19.0
Parts			
Breast	60	15 (25.0) [A]	15.7 - 37.3
Drum Stick	60	9 (15.0) [A]	7.9 - 26.3
Minced	30	3 (10.0) [A]	2.7 - 26.4
Thigh	60	13 (21.7) [A]	13.0 - 33.8
Whole	60	9 (15.0) [A]	7.9 - 26.3

582 [a]Values in columns within each variable that are followed by the same letter are not
583 significantly different ($P \geq 0.05$).

584

585

586 **Table 3.** *Salmonella* serovars isolated from different types of markets.

Serovars	No. (%) of isolates from wet markets	No. (%) of isolates from supermarkets	Total no. (%)
Saintpaul	3 (5.8)	14 (26.9)	17 (32.7)
Brancaster	6 (11.5)	5 (9.6)	11 (21.2)
Albany	6 (11.5)	0 (0)	6 (11.5)
Stanley	5 (9.6)	0 (0)	5 (9.6)
Agona	4 (7.7)	0 (0)	4 (7.7)
Typhimurium	3 (5.8)	0 (0)	3 (5.8)
Gaminara	2 (3.8)	0 (0)	2 (3.8)
Bovismorbificans	1 (1.9)	0 (0)	1 (1.9)
Give	1 (1.9)	0 (0)	1 (1.9)
Newport	0 (0)	1 (1.9)	1 (1.9)
Weltevreden	1 (1.9)	0 (0)	1 (1.9)
Total no. (%)	32 (61.4)	20 (38.4)	52 (100[a])

587 [a]Rounded up to 100

588

589 **Table 4.** Phenotypic resistance patterns of *Salmonella* isolates from raw chicken meat.

Resistance phenotype	Serovar (No. of isolates)	No. of antibiotic classes
Susceptible	Agona (4), Gaminara (2), Saintpaul (2), Newport (1), Weltevreden (1)	0
AMP	Saintpaul (4)	1
AMC-AMP	Saintpaul (1)	1
AMP-C	Typhimurium (1)	2
AMP-CRO	Saintpaul (3)	2
AMP-TE	Saintpaul (1), Typhimurium (1)	2
C-CIP-NA-TE	Give (1)	3
AMP-C-SXT-TE	Stanley (4), Bovismorbificans (1), Typhimurium (1), Brancaster (1)	4
AMC-AMP-C-CN-CRO	Saintpaul (1)	4
AMC-AMP-C-SXT-TE	Brancaster (1)	4
AMP-C-CN-SXT-TE	Saintpaul (5), Brancaster (1)	5
AMP-C-NA-SXT-TE	Albany (3), Stanley (1), Brancaster (1)	5
AMC-AMP-C-NA-SXT-TE	Brancaster (3), Albany (1)	5
AMP-C-CN-NA-SXT-TE	Brancaster (4), Albany (1)	6
AMP-AMC-C-CIP-CRO-NA-SXT-TE	Albany (1)	6

590 Abbreviations: AMC, Amoxicillin-clavulanate; AMP, Ampicillin; C, Chloramphenicol;

591 CIP, Ciprofloxacin; CN, Gentamicin; CRO, Ceftriaxone; NA, Nalidixic acid; SXT,

592 Sulfamethoxazole-trimethoprim; TE, Tetracycline.

593 **Table 5.** List of *gyrA* and *parC* mutations in quinolone and/or fluroquinolone resistant

594 isolates.

Sample	Serovar	NA	CIP	*gyrA* mutation	*parC* mutation
WB17[a]	Stanley	R	I	-	-
M22[b]	Brancaster	R	I	-	-
M24	Brancaster	R	I	-	-
T24[c]	Brancaster	R	S	-	-
W24[d]	Brancaster	R	I	-	-
WW8A	Brancaster	R	I	-	-
WW8B	Brancaster	R	I	-	-
WW28A	Brancaster	R	I	-	-
WW22	Brancaster	R	I	-	-
WB11	Give	R	R	Ser83Tyr	-
WB2	Albany	R	R	Asp87Asn	-
WB22	Albany	R	I	Asp87Asn	-
WB23	Albany	R	I	Asp87Asn	-
WB24	Albany	R	I	Asp87Asn	-
WB25	Albany	R	I	Asp87Asn	-
WT5	Albany	R	I	Asp87Asn	-

595 Abbreviations: NA, Nalidixic acid; CIP, Ciprofloxacin; R, Resistant; S, Susceptible, I,

596 Intermediate; [a]B denotes chicken breast meat sample while prefix W denotes wet market

597 sample; [b]M denotes mince chicken meat sample and the lack of prefix W denotes

598 supermarket sample; [c]T denotes thigh sample; [d]W denotes whole chicken sample.

599

600

601 **Figure Legends**

602

603 **Figure 1.** Neighbor-joining dendrogram based on concatenated nucleotide sequences of 7
604 house-keeping genes of *Salmonella* determined by multilocus sequence typing (MLST).
605 Bootstrap values of >50% shown on the branches are calculated after 1,000 replicates.

606

607 **Figure 2.** Frequency bar chart of percentage resistant *Salmonella* isolates to different
608 antibiotics.

609

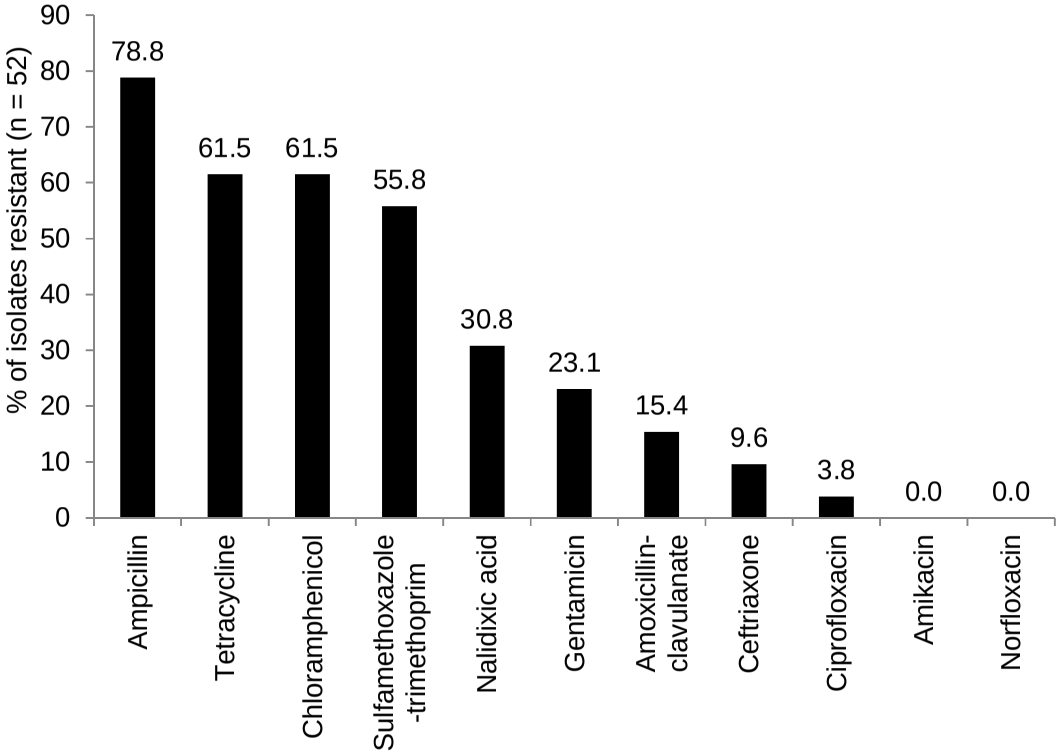
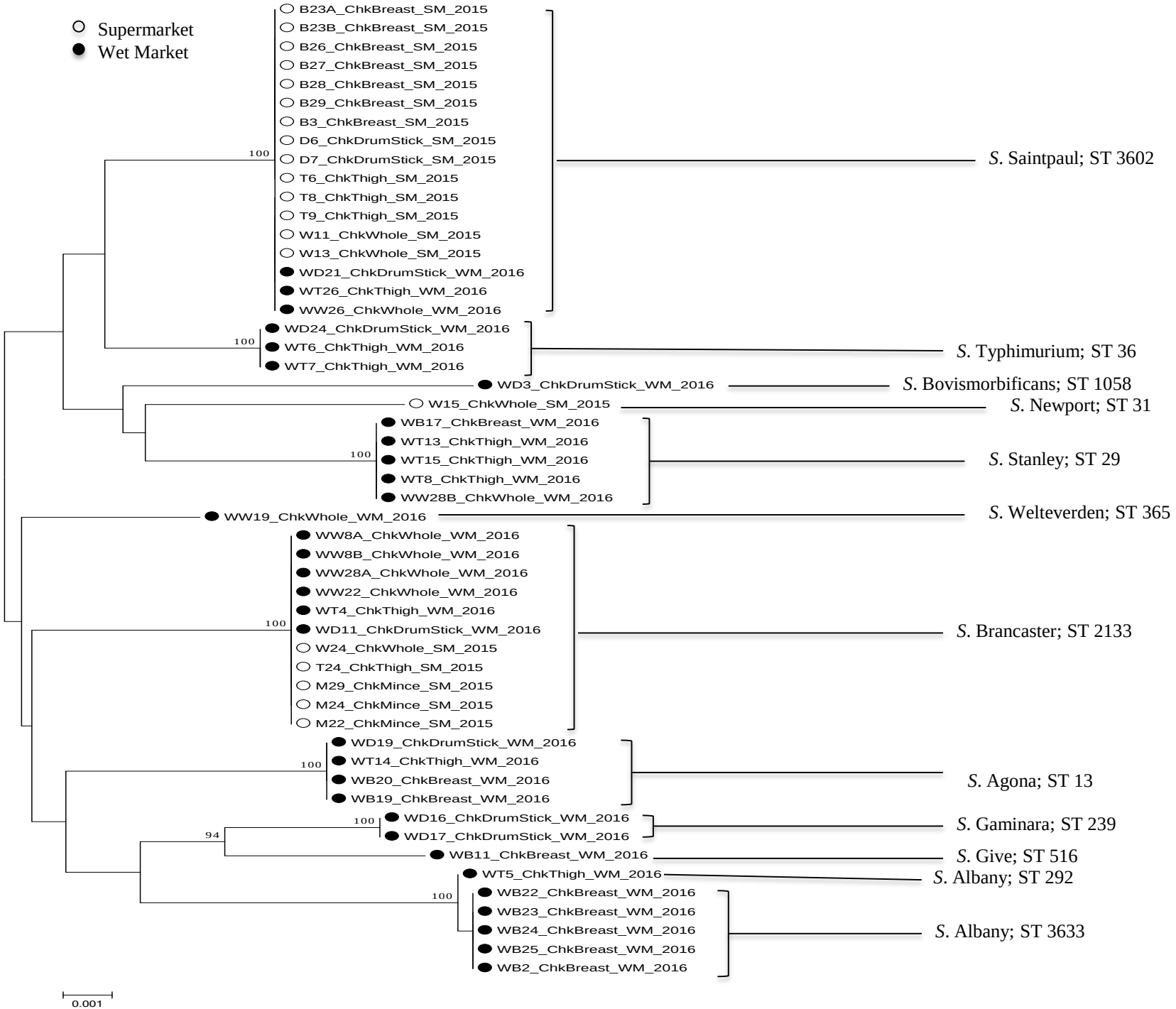


Fig. 2


Supermarket
Wet Market
B23A_ChkBreast_SM_2015
B23B_ChkBreast_SM_2015
B26_ChkBreast_SM_2015
B27_ChkBreast_SM_2015
B28_ChkBreast_SM_2015
B29_ChkBreast_SM_2015
B3_ChkBreast_SM_2015
D6_ChkDrumStick_SM_2015
D7_ChkDrumStick_SM_2015
T6_ChkThigh_SM_2015
T8_ChkThigh_SM_2015
T9_ChkThigh_SM_2015
W11_ChkWhole_SM_2015
W13_ChkWhole_SM_2015
WD21_ChkDrumStick_WM_2016
WT26_ChkThigh_WM_2016
WW26_ChkWhole_WM_2016
100
S. Saintpaul; ST 3602
WD24_ChkDrumStick_WM_2016
WT6_ChkThigh_WM_2016
WT7_ChkThigh_WM_2016
100
S. Typhimurium; ST 36
WD3_ChkDrumStick_WM_2016
S. Bovismorbificans; ST 1058
W15_ChkWhole_SM_2015
S. Newport; ST 31
WB17_ChkBreast_WM_2016
WT13_ChkThigh_WM_2016
WT15_ChkThigh_WM_2016
WT8_ChkThigh_WM_2016
WW28B_ChkWhole_WM_2016
100
S. Stanley; ST 29
WW19_ChkWhole_WM_2016
S. Welteverden; ST 365
WW8A_ChkWhole_WM_2016
WW8B_ChkWhole_WM_2016
WW28A_ChkWhole_WM_2016
WW22_ChkWhole_WM_2016
WT4_ChkThigh_WM_2016
WD11_ChkDrumStick_WM_2016
W24_ChkWhole_SM_2015
T24_ChkThigh_SM_2015
M29_ChkMince_SM_2015
M24_ChkMince_SM_2015
M22_ChkMince_SM_2015
100
S. Brancaster; ST 2133
WD19_ChkDrumStick_WM_2016
WT14_ChkThigh_WM_2016
WB20_ChkBreast_WM_2016
WB19_ChkBreast_WM_2016
100
S. Agona; ST 13
WD16_ChkDrumStick_WM_2016
WD17_ChkDrumStick_WM_2016
100
S. Gaminara; ST 239
WB11_ChkBreast_WM_2016
94
S. Give; ST 516
WT5_ChkThigh_WM_2016
S. Albany; ST 292
WB22_ChkBreast_WM_2016
WB23_ChkBreast_WM_2016
WB24_ChkBreast_WM_2016
WB25_ChkBreast_WM_2016
WB2_ChkBreast_WM_2016
100
S. Albany; ST 3633
0.001

Highlights

- *Salmonella* contamination was higher in the wet markets than supermarkets.
- *S*. Saintpaul (32.7%) was most prevalent, followed by *S*. Brancaster (21.2%).
- Multi-drug resistance rate of *Salmonella* was 60%.
- Two separate mutations in *gyrA* for quinolone resistance were found.