Characterization and Source Investigation of Multidrug-Resistant *Salmonella* Anatum from a Sustained Outbreak, Taiwan

Ye Feng, Yi-Jung Chang, Shih-Chuan Pan, Lin-Hui Su, Hsin-Chieh Li, Hsin-Ping Yang, Min-Jia Yu, Cheng-Hsun Chiu

An ongoing outbreak of multidrug-resistant *Salmonella enterica* serovar Anatum began in Taiwan in 2015. Pork and poultry were identified as vehicles for transmission. Contaminated meat contributed to the high rate of infections among children. Nearly identical *Salmonella* Anatum strains have been identified in the United Kingdom, the United States, and the Philippines.

Nontyphoidal *Salmonella* (NTS) is a major cause for foodborne diseases worldwide. In Taiwan, the ambient climate and flourishing pig-raising industry makes NTS infections rampant. As in other countries, salmonellosis was primarily caused by *Salmonella enterica* serovars Enteritidis and Typhimurium in Taiwan (*1*), but rare serovars such as *Salmonella* Goldcoast have appeared in recent years (*2*). Recommended antimicrobial treatment options for salmonellosis include fluoroquinolones and extended-spectrum cephalosporins (*1*). However, resistance to these antibiotics has been emerging in many countries, leading to increased disease prevalence, disease severity, and death and the requirement of last-line antimicrobial drugs (e.g., carbapenems) (*3*–*5*).

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Since 2015, northern Taiwan has seen an increase in *Salmonella* infections, caused by previously rare *Salmonella* Anatum. The infections were also reported in central Taiwan, indicating that this outbreak had already prevailed throughout the entire island (*6*). Co-resistance to ceftriaxone and ciprofloxacin are the main feature of the outbreak clone. Evidence from epidemiologic, laboratory, and supply-chain investigations identified raw pork and poultry as the vehicle for spread of this strain. More important, genomic comparisons against the global public database indicated that this clone has appeared in Europe, Asia, and America. Given the increasing globalization of foodstuffs, these findings prompt an urgent global sharing of whole-genome sequencing (WGS) data to facilitate disease surveillance and early recognition of international foodborne outbreaks (*7*,*8*).

The Study

Chang Gung Memorial Hospital is a main referral hospital for cities in northern Taiwan, including Taipei, New Taipei, and Taoyuan. The population in this region is ≈7 million. In 2012, the hospital's clinical microbiology laboratory launched a program to monitor the NTS serovars causing human infections. All *Salmonella* isolates from patients were collected and serotyped. Before 2015, very few *Salmonella* Anatum isolates were recovered, and most were susceptible to antimicrobial agents. Since then, an increase has been observed, peaking in 2017 (Figure 1, panel A). As of June 2019, a total of 319 nonrepetitive isolates have been identified; of these, 197 (61.8%) isolates were ceftriaxone-resistant (MIC ≥2 μg/mL), 301 (94.4%) were ciprofloxacin-resistant (MIC ≥0.12 μg/mL), and 197 (61.8%) were resistant to both. In addition, 292 (91.5%) isolates were resistant to chloramphenic, and 295 (92.5%) were resistant to trimethoprim/

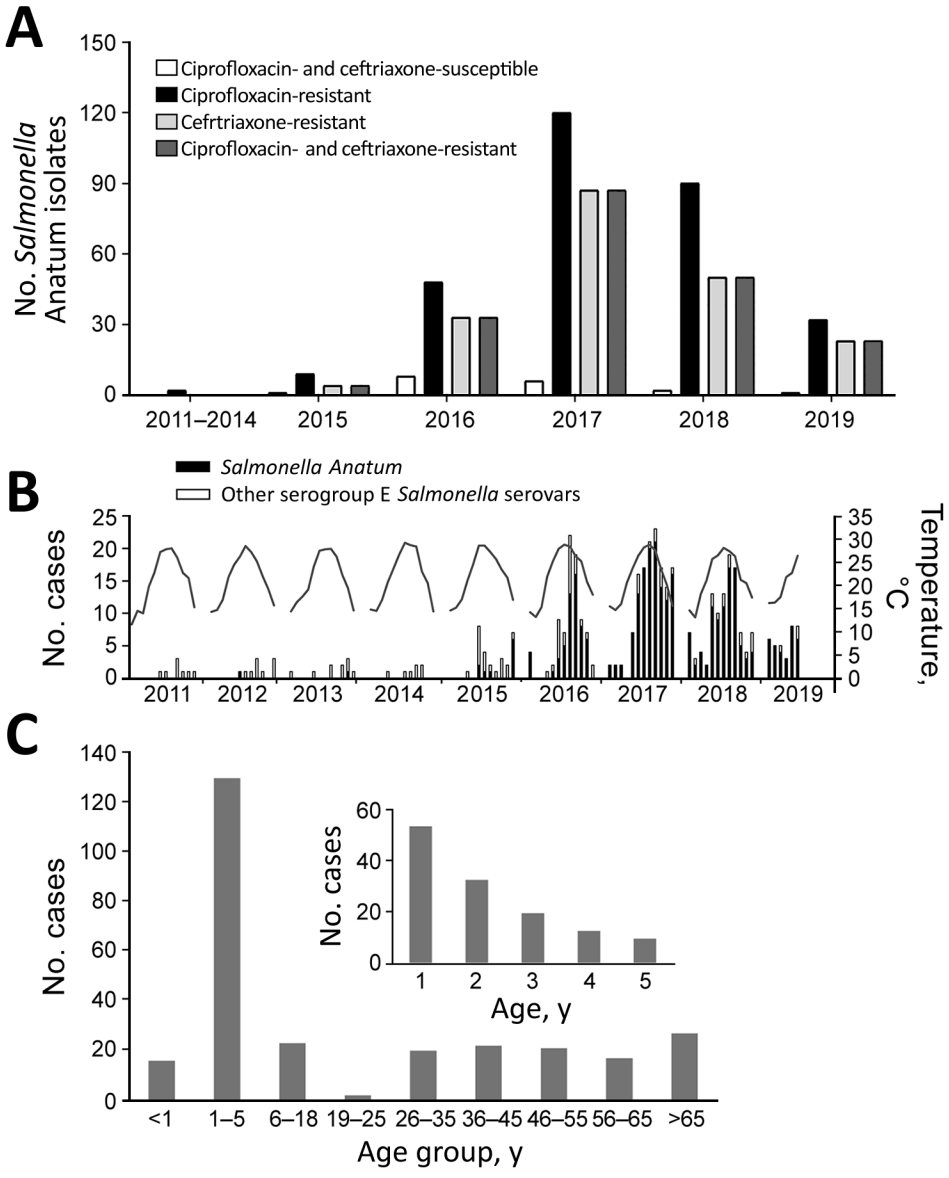

Figure 1. *Salmonella enterica* serotype Anatum infection and antimicrobial resistance, Taiwan. A) Antimicrobial resistance of the *Salmonella* Anatum isolates collected in Chang Gung Memorial Hospital. B) Monthly case number (bar plot) and temperature (line). C) Age distribution of patients diagnosed during 2015–2018.

sulfamethoxazole. A positive correlation was found between higher temperatures and the infections (r = 0.4; p<0.05) (Figure 1, panel B); however, no notable effects on *Salmonella.* Anatum infections have been associated with precipitation or humidity (r<0.3; p>0.05).

Detailed methods are described in the Appendix (https://wwwnc.cdc.gov/EID/article/26/12/20-0147-App1.pdf). We first reviewed the clinical and laboratory characteristics of 278 patients from 2015–2018. Most patients had acute gastroenteritis, whereas a few (14/278, 5%) had invasive diseases, such as bacteremia and sepsis. In terms of age distribution, the highest number of cases were in young children (Figure 1, panel C). Pediatric patients (n = 169) had significantly higher rates than adult patients (n = 109) for hospitalization (79.2% vs. 55.0%; p<0.05), diarrhea (89.9% vs. 68.8%; p<0.05), and fever (89.2% vs. 58.1%; p<0.05).

Multilocus sequence typing indicated that the entire collection of clinical *Salmonella* Anatum isolates belonged to sequence type 64. We randomly selected 54 clinical isolates for WGS (Appendix Table 1). Both core genome multilocus sequence typing and whole-genome single-nucleotide polymorphism analyses, performed by using the BacWGSTdb database (*9*), further divided these isolates into 3 clades (Figure 2, panel A, B). Clades I and II were more closely related to each other; their most recent common ancestor occurred >21 years ago.

Clade III was more distantly connected to these 2 clades. Typing based on PCR assay was performed on the unsequenced isolates. Clade I accounted for 95.6% (305/319) of all isolates, suggesting it was the cause of the outbreak. The isolates resistant to ceftriaxone, ciprofloxacin, or both clustered within clade I, whereas the isolates of clades II and III were more susceptible. Most of the clade I isolates harbored a 90-kb IncA/C plasmid carrying *bla*DHA-1 (encoding a class C β-lactamase) and *qnrB* (conferring resistance to quinolones). A conjugation assay demonstrated that this plasmid conferred ceftriaxone and ciprofloxacin resistance. In addition, 31 (9.7%) clinical isolates carried bla_{CMY-2}, which was located within a >100-kb IncI1 plasmid and also encoded a class C β-lactamase. These 31 isolates carried bla_{DHA-1} simultaneously. In 11 of them, the bla_{DHA-1}–carrying and bla_{CMY-2}–carrying plasmids were fused into 1 large plasmid (Figure 2, panel C).

By comparing these findings against sequences in GenBank, we found nearly identical genomic sequences for isolates in the United Kingdom, the

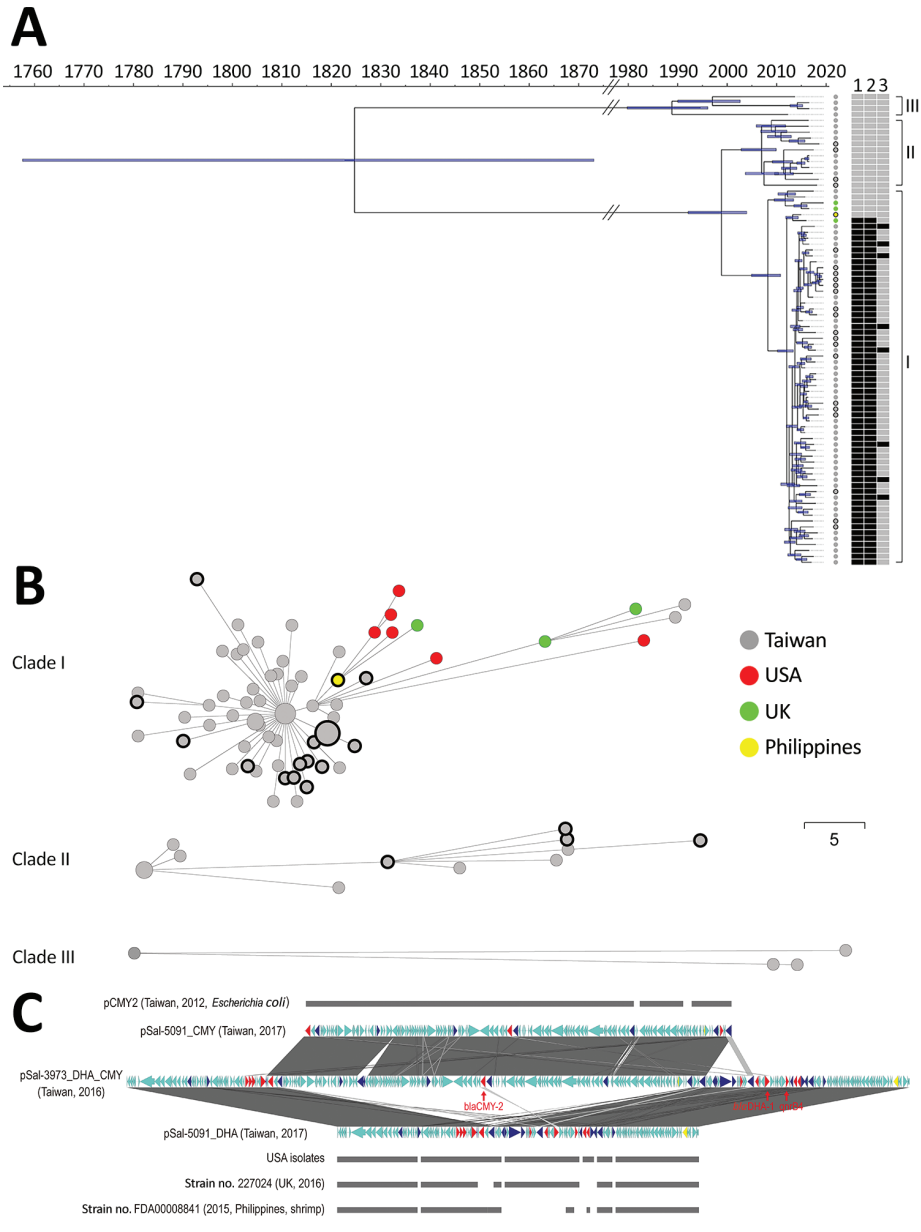

Figure 2. Genomic analysis of the outbreak caused by *Salmonella enterica* serotype Anatum, Taiwan. A) Dated phylogeny for *Salmonella* Anatum clinical isolates and food and environmental isolates. All isolates were divided into 3 clades, shown at right. The nodes' colors represent the geo source; nodes with black rings were from meat or the environment, and the remainder were derived from the patients. The right heatmap represents the presence (in black) or absence (in gray) of key antimicrobial-resistance genes (1, bla_{DHA-1}; 2, *qnrB4*; 3, bla_{CMY-2}). B) Minimal spanning tree based on alleles identified through core genome multilocus sequence typing. Dots with black circles represent food isolates; the others are clinical isolates. The collection date for the 6 US isolates in panel B was missing in GenBank and therefore not included in panel A. Scale bar indicates 5 single nucleotide polymorphisms. C) Gene structure of multidrug-resistant plasmids in *Salmonella* Anatum in Taiwan compared with international isolates. Two types of plasmids were identified in the clade I *Salmonella.* Anatum isolates in Taiwan. One carried bla_{CMY-2}, with its structure being shown by pSal-5091_CMY. A similar plasmid, pCMY2 (GenBank accession no. LC019731.1), is shown. The other carried bla_{DHA-1}; its structure is shown by pSal-5091_DHA. International isolates shown in the figure, whose genomes also were downloaded from GenBank (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/26/12/20-0147-App1.pdf), possess very similar plasmids. In certain isolates, the 2 plasmids can integrate into 1 large plasmid, with its structure shown by pSal-3973_DHA_CMY. Red genes represent antimicrobial-resistance genes; blue genes represent transposase/integrase genes; and yellow genes represent Inc-determinant genes.

United States, and the Philippines. The collection time for these isolates also occurred during 2015–2019, which nearly coincided with the outbreak in Taiwan. These international *Salmonella* Anatum isolates also carried the 90-kb IncA/C plasmid (Figure 2, panel A, C); therefore, they were likely ceftriaxone- and ciprofloxacin-resistant concomitantly. The only distinction of these international isolates was their lack of the bla_{CMY-2}–carrying plasmid. Accordingly, we speculated that the *Salmonella* Anatum clone had arrived in Taiwan through food trade and later acquired the bla_{CMY-2}–carrying plasmid.

To trace the source of *Salmonella* Anatum, we investigated food samples from supermarkets and traditional markets of 8 districts with high density of *Salmonella* patients in New Taipei City and Taoyuan City, Taiwan (Appendix Figure 1). A total of 11 *Salmonella* Anatum isolates were collected from pork, 4 from poultry, and 1 from beef in these regions (Appendix Table 2, Figure 1). WGS showed that they all belonged to clades I and II, providing strong evidence that raw meats were the outbreak vehicle. All 16 isolates harbored the bla_{DHA-1}–carrying IncA/C plasmid. Other *Salmonella* serovars also were detected in this investigation. The overall *Salmonella* isolation rate from retail meats was significantly higher in traditional markets than in the supermarkets ($p<0.001$) (Appendix Table 3). In Taiwan, pork in the supermarkets is usually provided through the cold transportation chain, whereas for traditional markets pork is usually provided through the traditional chain, with notable differences. Temperatures were much lower in the cutting factory and butcher shop in the cold chain than in the traditional chain (Appendix Figure 2). Furthermore, pork was wrapped by plastic tissue and bags in the cold chain, but the traditional chain did not do any wrapping or packaging during transportation.

To clarify the contradictory findings that most infections occurred in young children even though pork is not a major food for infants, we conducted a questionnaire survey among parents of 20 infants (<1 year of age) with NTS infections and 80 parents of infants without (controls) (Appendix). Parents of the infected infants more often touched, rinsed, and cooked meat before feeding other foods to their infants (Appendix Table 4). Moreover, these parents were more willing to purchase meat from traditional markets rather than supermarkets. A possibility is that they bought meat from the traditional markets, then their frequent rinsing flushed the *Salmonella* on the surface of the meats, cutting boards and knives, and sinks, and finally onto fresh vegetables, fruit, and other ready-to-eat foods that were cross-contaminated and reached the infants through parents or other caregivers. This transmission mode is of particular importance in infants and has already been reported for other bacterial pathogens such as *Yersinia enterocolitica* (*10*).

Conclusions

Our study sought to describe an outbreak in Taiwan caused by a multidrug-resistant *Salmonella* Anatum clone. The questionnaire and supply-chain investigations we conducted found that the infection cases were closely associated with improper packaging during transportation and unhygienic food handling in the customers' kitchen. The high similarity of genomic sequence between the Taiwan isolates and international isolates indicates the global dissemination of this clone and highlights the public health value of multicountry sharing of epidemiologic, trace-back, microbiologic, genomic, and food trade data.

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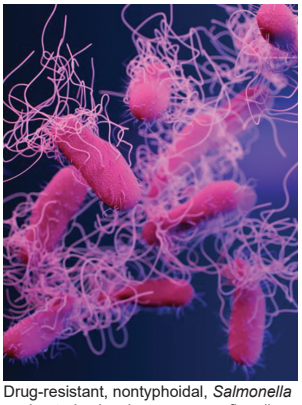
etymologia

Salmonella [sal''mo-nel'ə]

Daniel F. M. Monte, Fábio P. Sellera

Named in honor of Daniel Elmer Salmon, an American veterinary pathologist, *Salmonella* is a genus of motile, gram-negative bacillus, nonspore-forming, aerobic to facultatively anaerobic bacteria of the family *Enterobacteriaceae*. In 1880, Karl Joseph Eberth was the first to observe *Salmonella* from specimens of patients with typhoid fever (from the Greek *typhōdes* [like smoke; delirious]), which was formerly called *Eberthella typhosa* in his tribute. In 1884, Georg Gaffky successfully isolated this bacillus (later described as *Salmonella* Typhi) from patients with typhoid fever, confirming Eberth's findings. Shortly afterward, Salmon and his assistant Theobald Smith, an American bacteriologist, isolated *Salmonella* Choleraesuis from swine, incorrectly assuming that this germ was the causative agent of hog cholera. Later, Joseph Lignières, a French bacteriologist, proposed the genus name *Salmonella* in recognition of Salmon's efforts.

With a complicated taxonomy, the genus *Salmonella* is currently classified into 2 species (*S. enterica* and *S. bongori*), encompassing 2,659 serotypes based on somatic O and H flagellar antigens as specified in the Kauffmann–White–Le Minor scheme. *S. enterica* is divided into 6 subspecies: e*nterica, salamae, arizonae, diarizonae, houtenae,* and *indica*. Arguably, this zoonotic pathogen remains one of the most pressing global concerns. It causes a spectrum of diseases in several hosts, and there is much to be learned and deciphered about its continuous evolution.



Drug-resistant, nontyphoidal, *Salmonella* sp. bacteria showing numerous flagella. Taken from Antibiotic Resistance Threats in the United States, 2019 (AR Threats Report); Centers for Disease Control and Prevention. Illustration: James Archer/CDC, 2019.

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Appendix

Supplementary Methods

Patients and Setting

Chang Gung Memorial Hospital (CGMH) is a main referral hospital for cities in northern Taiwan, including Taipei, New Taipei, and Taoyuan. The population in this region is approximately seven million. The Clinical Microbiology Laboratory has launched a program to monitor the serovars of NTS causing human infections since 2012. All *Salmonella* isolates from patients were collected and serotyped. Antimicrobial susceptibility testing was performed using the disc diffusion method specified in the Clinical and Laboratory Standards Institute (CLSI) guidelines (*1*). *S.* Anatum is a relatively uncommonly recorded serovar for human infections. Prior to 2015, very few *S.* Anatum isolate was recovered and most of the isolates were susceptible to antimicrobial agents. However, since 2015, culture-confirmed infections caused by *S.* Anatum has been increasing (Figure 1, https://wwwnc.cdc.gov/EID/article/26/12/20-0147-F1.htm). A review on the clinical manifestations of the patients with *S.* Anatum infection from 2015 to 2018 was carried out. This study was approved by the Institutional Review Board of CGMH (201601804B0 and 201702155B0).

Serogrouping and serotyping was conducted as described previously (*2*). The MIC of CIP and CRO on these isolates was determined by E-test and interpreted according to the recommendations provided by CLSI (*1*).

To trace the source of *S.* Anatum, we investigated food samples from supermarkets and traditional markets of eight districts with high density of *Salmonella* patients in New Taipei City, and Taoyuan City in Taiwan (Appendix Figure 1). All the *Salmonella* isolates derived from food

samples were further examined for their serogroups, serovars, and their antimicrobial susceptibility to CRO and CIP.

Meteorological data, including temperature, humidity and precipitation in Northern Taiwan, were collected from the Taiwan Central Weather Bureau, which is available at: https://e-service.cwb.gov.tw/HistoryDataQuery/index.jsp. The correlation and lag effect of case number and temperature were analyzed using bivariate correlation and linear regression softwares in IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY), and significance was set at $p < 0.05$.

Meat Processing and Transport

To clarify the discrepancy of *Salmonella* detection rate between different markets, and to further investigate suspicious contamination nodes during meat processing and transportation, we designed an experiment to contrast cold chain with traditional chain transportation of the meat. Four nodes at each of the transportation lines were investigated: slaughter houses (samples obtained from the carcass surfaces), cutting factories and pre-cooling chambers, where the pig carcass were cut into large pieces (samples from the environmental and meat surfaces),transport vehicles (samples from the environment), and butcher shops, where the large pork chunks were cut into small pieces for consumer purchase (samples from the environment and meat surfaces). We collected carcass surface samples and environment samples from floor, hook, conveyor belt, meat grinder, basket, kitchen knife, chopping board and workers' hands surface in slaughterhouse, cutting factory, transport vehicle and butcher shop. Each sampling surface area of the carcass and environment samples was 100 cm^2. In addition, each of the meat samples was collected over 25 g in cutting factory and butcher shop. Meat isolation and identification followed the method of Taiwan Food and Drug Administration. Carcass surface and environment sampling followed USDA Laboratory Guidebook (https://www.fsis.usda.gov) with a sensitivity of 1.4×10^1 CFU/100 cm^2. All investigated samples were collected with sterilized sponges, bags, gloves and templates (Nasco Whirl-Pak, USA).

Whole-Genome Sequencing and Conjugation

A total of 76 isolates were subjected to whole genome sequencing (WGS), that was performed using the Illumina Miseq platform (Illumina, CA, USA) and/or MinION Sequencer (Nanopore, Oxford, UK). The genome assembly and comparative analysis followed the methods

as described previously (*2*). The genomic sequences were deposited into the GenBank database, with the accession numbers being listed in Appendix Table 1. The NCBI Pathogen Detection Service (https://www.ncbi.nlm.nih.gov/pathogens) was searched for *S.* Anatum genomes that were highly similar to the genome sequences collected in Taiwan. Multilocus sequence typing was performed on all *S.* Anatum isolates (*2*,*3*).

To investigate the transferability of the resistance plasmids identified in the genome sequencing, we carried out a conjugation assay using *E. coli* J53 (a sodium-azide-resistant strain) as the recipient and outbreak *S.* Anatum isolates as the donor (Sal-4377 and Sal-4162). The conjugation assay was conducted following the protocol described in a previous study (*2*).

Questionnaire Investigation

Caregivers of children with culture-confirmed *S.* Anatum infection were invited to complete a questionnaire. The questionnaire is designed based on a WHO protocol (https://www.who.int/immunization/diseases/rotavirus/generic_protocols/en/). The total number of the questionnaire completed was 100, and the ratio of the patient to healthy controls was 1:4, namely 20 children in the patient group and 80 in the healthy group. The healthy children were recruited from well-baby clinics. Ages (<1y) were matched between the case and control groups. Chi-square test was used to analyze all questionnaire data using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY). The false-discovery rate (FDR) correction was made for multiple comparisons, with 0.1 as the significance threshold.

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Appendix Table 1. Accession numbers of the genomic sequences sequenced and analyzed in this study

Strain/Isolate	Collection Date	Country/ Region	Source	Accession	Note
GB10	2019/7/3	Taiwan	food/environment	WHYQ00000000	sequenced in this study
GC64–2	2019/7/3	Taiwan	food/environment	WHYP00000000	sequenced in this study
GC66–1	2019/7/3	Taiwan	food/environment	WHYO00000000	sequenced in this study
GC67–2	2019/7/3	Taiwan	food/environment	WHYN00000000	sequenced in this study
GC68–1	2019/7/3	Taiwan	food/environment	WHYM00000000	sequenced in this study
M-2589	2018/8/21	Taiwan	food/environment	WHYL00000000	sequenced in this study
M-2592	2018/8/21	Taiwan	food/environment	WHYK00000000	sequenced in this study
M-3471	2018/3/31	Taiwan	food/environment	CP045458-CP045460	sequenced in this study
M-3851	2017/6/15	Taiwan	food/environment	CP045461-CP045463	sequenced in this study
M-3853	2017/6/15	Taiwan	food/environment	WHYJ00000000	sequenced in this study
M-4763	2019/7/3	Taiwan	food/environment	WHYI00000000	sequenced in this study
M-4847	2018/10/3	Taiwan	food/environment	WHYH00000000	sequenced in this study
M-4949	2018/1/11	Taiwan	food/environment	WHYG00000000	sequenced in this study
M-5351	2017/8/3	Taiwan	food/environment	WHYF00000000	sequenced in this study
M-5360	2017/8/3	Taiwan	food/environment	CP045509-CP045512	sequenced in this study
M-5365	2017/8/3	Taiwan	food/environment	WHYE00000000	sequenced in this study
M-6525	2019/7/25	Taiwan	food/environment	WHYD00000000	sequenced in this study
M-6697	2018/4/16	Taiwan	food/environment	WHYC00000000	sequenced in this study
M-6699	2018/4/16	Taiwan	food/environment	WHYB00000000	sequenced in this study
M-7537	2018/8/2	Taiwan	food/environment	WHYA00000000	sequenced in this study
M-9196	2017/8/18	Taiwan	food/environment	WHXZ00000000	sequenced in this study
M-9750	2019/6/13	Taiwan	food/environment	WHXY00000000	sequenced in this study
Sal-1135	2012/6/22	Taiwan	human	CP045464	sequenced in this study
Sal-2097	2013/11/1	Taiwan	human	CP045465	sequenced in this study
Sal-2955	2015/6/13	Taiwan	human	WHXX00000000	sequenced in this study
Sal-3348	2015/12/5	Taiwan	human	WHXW00000000	sequenced in this study
Sal-3381	2015/12/19	Taiwan	human	WHXV00000000	sequenced in this study
Sal-3386	2015/12/21	Taiwan	human	WHXU00000000	sequenced in this study
Sal-3389	2015/12/21	Taiwan	human	WHXT00000000	sequenced in this study
Sal-3343	2015/12/3	Taiwan	human	WHXS00000000	sequenced in this study
Sal-3660	2016/6/29	Taiwan	human	WHXR00000000	sequenced in this study
Sal-3805	2016/7/23	Taiwan	human	WHXQ00000000	sequenced in this study
Sal-3824	2016/7/28	Taiwan	human	WHXP00000000	sequenced in this study
Sal-3892	2016/8/17	Taiwan	human	WHXO00000000	sequenced in this study
Sal-3897	2016/8/20	Taiwan	human	WHXN00000000	sequenced in this study
Sal-3930	2016/9/1	Taiwan	human	WHXM00000000	sequenced in this study
Sal-3944	2016/9/5	Taiwan	human	WHXL00000000	sequenced in this study
Sal-3948	2016/9/6	Taiwan	human	CP045513-CP045514	sequenced in this study
Sal-3973	2016/9/15	Taiwan	human	CP045466-CP045467	sequenced in this study
Sal-3985	2016/9/10	Taiwan	human	WHXK00000000	sequenced in this study
Sal-3991	2016/9/19	Taiwan	human	WHXJ00000000	sequenced in this study
Sal-3993	2016/9/11	Taiwan	human	WHXI00000000	sequenced in this study
Sal-3997	2016/9/13	Taiwan	human	WHXH00000000	sequenced in this study
Sal-4162	2016/10/25	Taiwan	human	WHXG00000000	sequenced in this study
Sal-4179	2016/10/20	Taiwan	human	WHXF00000000	sequenced in this study

Strain/Isolate	Collection Date	Country/ Region	Source	Accession	Note
Sal-4221	2016/11/23	Taiwan	human	WHXE00000000	sequenced in this study
Sal-4295	2017/1/11	Taiwan	human	CP045515	sequenced in this study
Sal-4377	2017/3/26	Taiwan	human	WHXD00000000	sequenced in this study
Sal-4420	2017/5/5	Taiwan	human	WHXC00000000	sequenced in this study
Sal-4478	2017/5/22	Taiwan	human	WHXB00000000	sequenced in this study
Sal-4494	2017/5/31	Taiwan	human	WHXA00000000	sequenced in this study
Sal-4499	2017/6/2	Taiwan	human	WHWZ00000000	sequenced in this study
Sal-4518	2017/6/4	Taiwan	human	WHWY00000000	sequenced in this study
Sal-4550	2017/6/16	Taiwan	human	WHWX00000000	sequenced in this study
Sal-4567	2017/6/21	Taiwan	human	WHWW00000000	sequenced in this study
Sal-4583	2017/6/28	Taiwan	human	WHWV00000000	sequenced in this study
Sal-4627	2017/7/14	Taiwan	human	WHWU00000000	sequenced in this study
Sal-4698	2017/8/3	Taiwan	human	WHWT00000000	sequenced in this study
Sal-4737	2017/8/17	Taiwan	human	CP045516-CP045517	sequenced in this study
Sal-4762	2017/8/21	Taiwan	human	WHWS00000000	sequenced in this study
Sal-4873	2017/9/15	Taiwan	human	WHWR00000000	sequenced in this study
Sal-4995	2017/10/16	Taiwan	human	WHWQ00000000	sequenced in this study
Sal-5067	2017/11/16	Taiwan	human	WHWP00000000	sequenced in this study
Sal-5091	2017/11/24	Taiwan	human	CP045518-CP045521	sequenced in this study
Sal-5131	2017/12/6	Taiwan	human	WHWO00000000	sequenced in this study
Sal-5147	2017/12/14	Taiwan	human	WHWN00000000	sequenced in this study
Sal-5186	2018/1/10	Taiwan	human	WHWM00000000	sequenced in this study
Sal-5191	2018/1/10	Taiwan	human	WHWL00000000	sequenced in this study
Sal-5196	2018/1/19	Taiwan	human	WHWK00000000	sequenced in this study
Sal-5200	2018/1/22	Taiwan	human	WHWJ00000000	sequenced in this study
Sal-5217	2018/2/22	Taiwan	human	WHWI00000000	sequenced in this study
Sal-5226	2018/2/27	Taiwan	human	WHWH00000000	sequenced in this study
Sal-5240	2018/3/16	Taiwan	human	WHWG00000000	sequenced in this study
Sal-5242	2018/3/19	Taiwan	human	WHWF00000000	sequenced in this study
Sal-5328	2018/5/21	Taiwan	human	WHWE00000000	sequenced in this study
Sal-5379	2018/6/7	Taiwan	human	WHWD00000000	sequenced in this study
795421	Aug. 2019	United Kingdom	human	AAKCLH01	downloaded from public database
300680	Sep. 2016	United Kingdom	human	AAHNES01	downloaded from public database
PNUSAS010879	Missing	USA	human	AAEHEA01	downloaded from public database
PNUSAS011492	Missing	USA	human	AAGSRM01	downloaded from public database
PNUSAS038936	Missing	USA	human	AAIICK01	downloaded from public database
PNUSAS051059	Missing	USA	human	AADNYC01	downloaded from public database
PNUSAS051057	Missing	USA	human	AADTUF01	downloaded from public database
PNUSAS068759	Missing	USA	human	AADAOT01	downloaded from public database
FDA00008841	2015/2/20	Philippines	food/environment	AAGLTB01	downloaded from public database
227024	Feb. 2016	United Kingdom	human	AAHNEA01	downloaded from public database

Appendix Table 2. *Salmonella* serovars isolated from 438 food samples obtained from traditional markets and supermarkets in northern Taiwan in 2017–2019.

Food	Positive Rate	Serotype (n)
Raw Pork	50.7% (75/148)	S. Agona (10); *S.* Anatum (11); *S.* Corvallis (1); *S.* Derby (16); *S.* Give (5); *S.* Goldcoast (1); *S.* Kentucky (3); *S.* Livingstone (3); *S.* London (8); *S.* Mbandaka (2); *S.* Muenster (4); *S.* Newport (2); *S.* Potsdam (1); *S.* Rissen (1); *S.* Typhimurium (3); *S.* Weltevreden (4)
Raw Chicken	36.6% (34/93)	*S.* Albany (6); *S.* Anatum (4); *S.* Brancaster (3); *S.* Derby (1); *S.* Enteritidis (4); *S.* Goldcoast (1); *S.* Kentucky (6); *S.* Livingstone (2); *S.* Muenster (2); *S.* Schwarzengrund (2); *S.* Thompson (1); *S.* Typhimurium (2)
Raw Beef	3.7% (1/27)	*S.* Anatum (1)
Raw Duck	100% (1/1)	*S.* Albany (1)
Egg	0% (0/46)	ND
Vegetable	5.2% (5/96)	*S.* Albany (1); *S.* Derby (2); *S.* Kaitaan (1); *S.* Zigong (1)
Seafood	6.7% (1/15)	*S.* Albany (1)
Fruit	0% (0/12)	ND

ND, not detected.

Appendix Table 3. *Salmonella* isolation rate between supermarkets and traditional markets in northern Taiwan in 2017–2019*

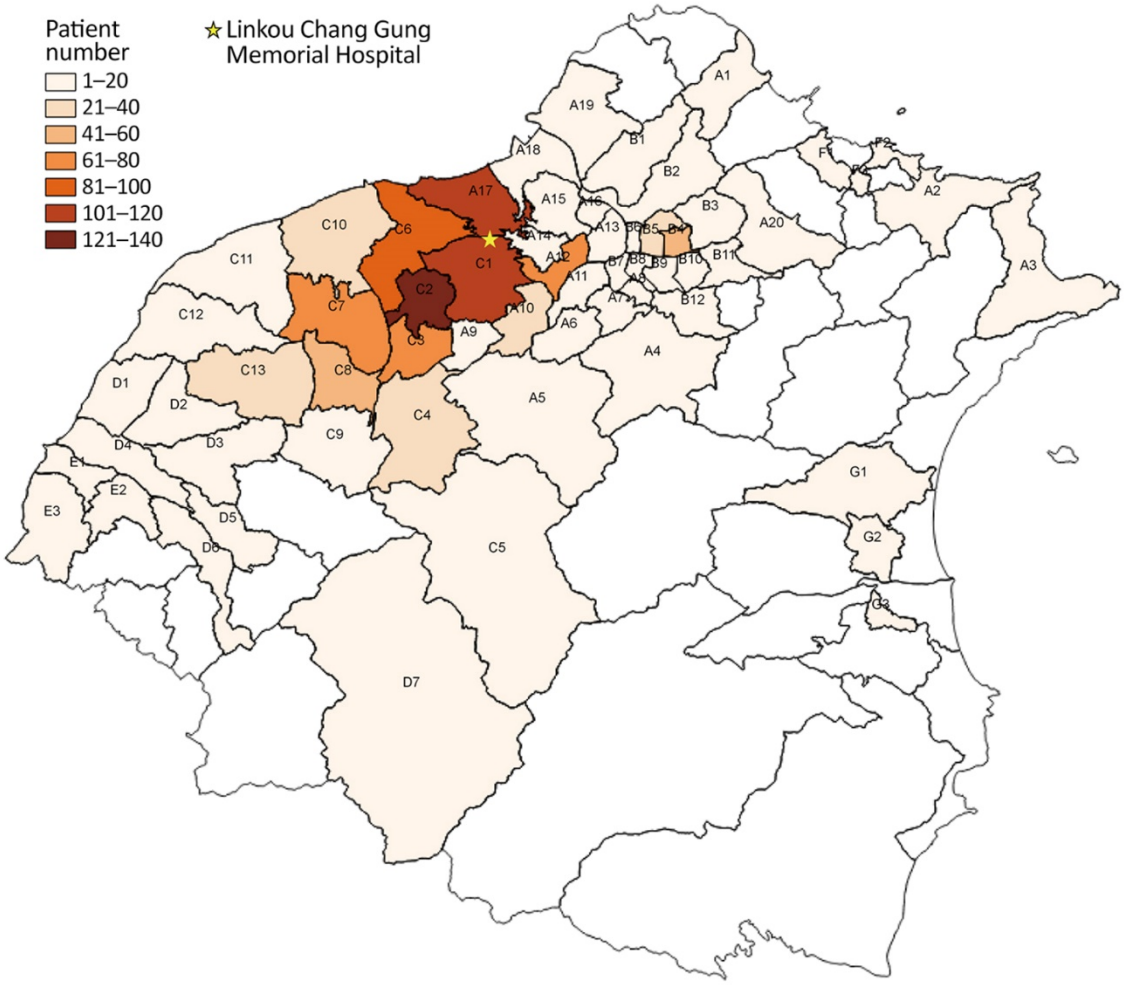
Sample	Source (n)	Positive (n)	Negative (n)	Positive Rate (%)	*P*-value
Pork	Supermarket (36)	4	32	11.1	<0.001
	Traditional Market (112)	71	41	63.4	
Chicken	Supermarket (36)	7	29	19.4	0.006
	Traditional Market (57)	27	30	47.4	
Duck	Supermarket (0)	ND	ND	ND	NA
	Traditional Market (1)	1	0	100	
Beef	Supermarket (12)	0	12	0	NA
	Traditional Market (15)	1	14	0	
Seafood	Supermarket (8)	0	8	0	0.268
	Traditional Market (7)	1	6	14.3	
Egg	Supermarket (11)	0	11	0	NA
	Traditional Market (35)	0	35	0	
Vegetable	Supermarket (37)	0	37	0	0.069
	Traditional Market (59)	5	54	8.5	
Fruit	Supermarket (4)	0	4	0	NA
	Traditional Market (8)	0	8	0	

*ND, not detected; NA, not applicable.

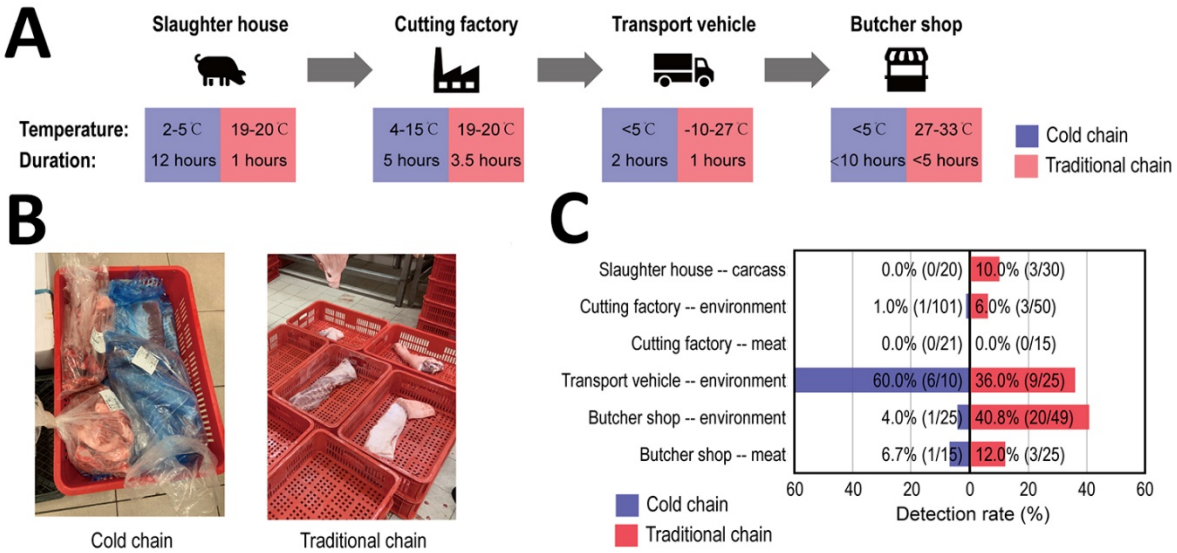
Appendix Table 4. Case-control survey for *Salmonella*-infected infants under 1 y of age in Chang Gung Memorial Hospital, Taiwan in 2019.

	Salmonella (n = 20)		Healthy (n = 80)						
Items	n	%	n	%	Odds Ratio	95% Low	95% High	p-value	FDR*
Contact									
Share toys with other people	4	20%	13	16%	1.288	0.371	4.481	0.69	0.794
Share pillow, comforter or mattress with other people	10	50%	35	44%	1.286	0.482	3.431	0.615	0.755
Surroundings									
Purchase bulk eggs without washing	11	55%	19	24%	3.924	1.414	10.886	0.006	0.081
Traditional market as the major pork shopping place	11	55%	20	25%	3.667	1.328	10.127	0.009	0.081
Home cooking/Vegetable	18	90%	59	74%	3.203	0.684	14.994	0.122	0.302
Home cooking/Rice, noodle, other grains	18	90%	61	76%	2.803	0.596	13.194	0.177	0.376
Home cooking/Pork	14	70%	38	48%	2.579	0.900	7.386	0.072	0.249
Cook raw food and delicatessen with the same chop board	10	50%	23	29%	2.478	0.910	6.746	0.071	0.249
Main caregiver are grandparents or other elders	8	40%	17	21%	2.471	0.871	7.009	0.083	0.249
Traditional market as the major egg shopping place	8	40%	20	25%	2.000	0.716	5.590	0.181	0.376
Home cooking/Eggs	13	65%	46	58%	1.373	0.495	3.807	0.542	0.752
Diet									
Rinse or wash raw meat before cooking	18	90%	50	63%	5.400	1.170	24.923	0.018	0.122
Process raw meat before feeding infants	10	50%	15	19%	4.333	1.530	12.271	0.004	0.081
Eat banana	5	25%	8	10%	3.000	0.861	10.452	0.074	0.249
Eat pork	6	30%	15	19%	1.857	0.613	5.629	0.269	0.484
Eat apple	7	35%	18	23%	1.855	0.644	5.343	0.248	0.478
Use milk powder, rice cereal or malt extract in 30 d	5	25%	14	18%	1.571	0.490	5.037	0.444	0.749
Wash and air-dry scoop after feeding milk powder, rice cereal or malt extract	11	55%	37	46%	1.420	0.531	3.802	0.484	0.752
Eat rice cereal	4	20%	12	15%	1.417	0.404	4.973	0.585	0.752
Infants need to be fed by caregivers	15	75%	55	69%	1.364	0.446	4.167	0.585	0.752
Eat shrimp	7	35%	23	29%	1.334	0.472	3.770	0.585	0.752
Drinking water after water filter and boiling	5	25%	17	21%	1.235	0.393	3.882	0.717	0.794
Frequency of handwashing before feeding infants (>75%)	6	30%	21	26%	1.204	0.410	3.540	0.735	0.794
Diet/Dairy Products									
Milk powder	17	85%	66	83%	1.202	0.310	4.665	0.790	0.820
Breast milk	3	15%	26	33%	0.367	0.099	1.363	0.123	0.302
Purchase Source									
Traditional markets	14	70%	34	43%	3.157	1.100	9.058	0.028	0.151
Supermarkets	8	40%	34	43%	0.902	0.332	2.448	0.839	0.839

*FDR, False Discovery Rate.



Appendix Figure 1. Food samples surveyed for *Salmonella* from supermarkets and traditional markets of eight districts with higher density of *Salmonella* infection in New Taipei City and Taoyuan City of northern Taiwan. *S.* Anatum were isolated from traditional markets and supermarkets of Linkou District (A17) and Xinzhuang District (A12), and from traditional markets of Taoyuan District (C2), Zhongli District (C7), and Guishan District (C1).



Appendix Figure 2. A comparison of *Salmonella* detection rates between the cold chain and traditional chain. A) Differences of temperature and transportation time between the two transportation chains. B) Different packaging manners employed during transportation. C) A comparison of detection rates of *Salmonella* spp. between the cold and traditional transportation chains.