**SUPPLEMENTARY MATERIAL**

# Whole-genome sequencing analysis of *Salmonella* serotypes recovered longitudinally from broiler production, processing, and retailing in Trinidad and Tobago

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## Material and methods

### Study design

The study design was 3-cross-sectional: at a selected broiler farm, processing plant, and supermarket outlet using a batch sampling approach. This entailed following broiler chickens reared on a broiler farm, following the same batch of chickens to slaughter at a processing plant, and sampling chilled carcasses from the same processing plant at a supermarket. The samples were collected longitudinally.

### Selection of broiler farm to use in the study

Initially, samples were collected at 4 broiler farms, comprising 2 each from 2 processors in the country, where the chicken were less than 10 days old. On each of the 4 farms, cloacal swabs were randomly collected from chicken aged 10 to 25 days and at day 35 from a selected pen to determine their *Salmonella* status.

**Collection of samples**

Figure S1 shows the types and number of samples collected at the 3 production levels. Overall, 32, 40, and 10 samples were collected from the broiler farm, processing plant, and supermarket, respectively. All samples were collected as previously described (Khan et al. 2018; 2021; 2022)

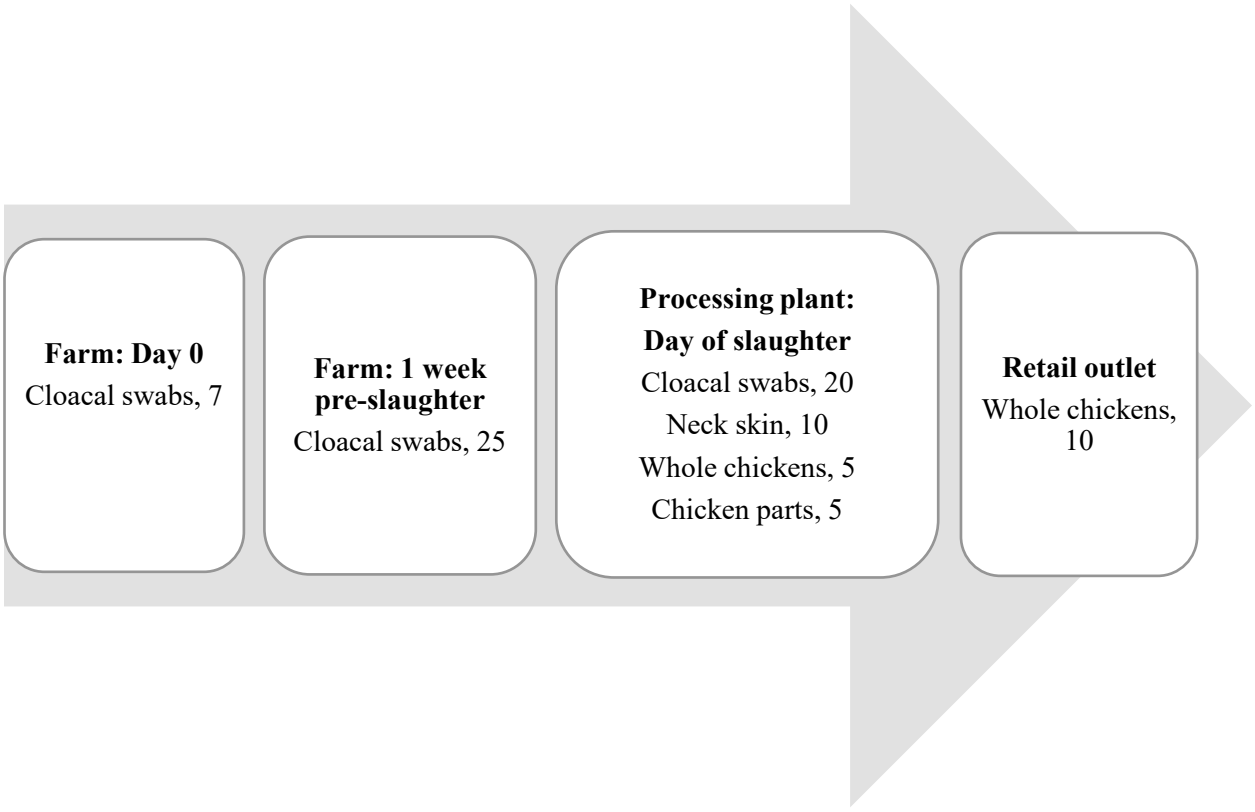


**Fig. S1** Schematics showing the number of samples collected from the positive farm, at processing plant and retail outlet

**Processing of samples, isolation, and identification of *Salmonella***

The methods used for *Salmonella* isolation, and phenotypic identification from farms, processing plants, and retail outlets were as previously described (Khan et al. 2018; 2021; 2022).

**Determination of antimicrobial resistance**

49 isolates of *Salmonella* representative of the various sources and enrichment media/plating were subjected to disk diffusion antimicrobial susceptibility to 8 antimicrobial agents, as previously described (Clinical and Laboratory Standards Institute 2017). The antimicrobial agents (Difco, BD) and concentrations used were amoxicillin-clavulanic acid (AMC, 30 µg), doxycycline (DO, 30 µg), ceftriaxone (CRO, 30 µg), gentamicin (CN, 10 µg), kanamycin (K, 30 µg), chloramphenicol (C, 30 µg), sulfamethoxazole-trimethoprim (SXT, 23.75 and 1.25 µg), and ciprofloxacin (CIP, 5 µg). The antimicrobial agents selected for the study were based on their being readily available and used in the poultry industry in Trinidad and Tobago.

**DNA extraction and sequencing**

13 non-duplicate isolates of *Salmonella*, representative of the various sources, were subjected to WGS. Genomic DNA was extracted using the Maxwell RSC cultured cells DNA kit with a Maxwell RSC instrument (Promega Corporation, Madison, WI, USA), following the manufacturer's protocols for Gram-negative bacteria with additional RNase treatment. DNA concentrations were measured with a Qubit fluorometer (Life Technologies, Carlsbad, CA, USA), standardized to 0.2 ng/µL, and the DNA was stored at 4 °C before library preparation. Whole-genome sequencing (WGS) of the *Salmonella* isolates was performed by the Food and Drug Administration (FDA): Center for Food Safety and Applied Nutrition genomics laboratory (FDA-CFSAN), Maryland, USA. The WGS data was generated on an Illumina MiSeq using 2 x 250 bp paired-end chemistry (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions, at 50–150X coverage. According to the manufacturer's instructions, the libraries were constructed using 100 ng of genomic DNA using the Illumina DNA Prep (M) tagmentation kit (Illumina Inc., San Diego, CA, USA).

**Genomic data analysis and in silico determination of genetic elements**

Quality control including adapter removal from the raw data was done using BBDuk (v.37.90; https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbduk-guide/; sourceforge.net/projects/bbmap/). SPAdes v.3.12.0 (Bankevich

et al. 2012) was used to create a *de novo* assembly of each isolate. Only contigs larger than 500 bp were retained for further analysis. Serovar prediction was made using command-line version of SISTR (Yoshida et al. 2016) (Version: sistr_cmd v.1.1.1). CARD (The Comprehensive Antibiotic Resistance Database) (Alcock et al. 2020) was also used to assign antimicrobial resistance. This was done with the predicted genes (amino acid format) from Prodigal using NCBI-blast-2.9.0+. Results were filtered for the top hit with 100 % identity and 100 % alignment length. VFDB (Liu et al. 2019) was used to assign virulence factors. This was done with the predicted genes (amino acid format) from Prodigal using NCBI-blast-2.9.0+. Results were filtered for the top hit with 100 % identity and 100 % alignment length.

**Determination of phylogeny**

Maximum likelihood phylogenies of all serovars from this study and reference genomes (completely closed genomes) for each serovar, downloaded from NCBI, were calculated using core genome alignment. Reference genomes used for each serovar was as follows: Infantis_strain_119944 (Accession: NZ_CP047881), Kentucky_strain_CVM_30177 (Accession: NZ_CP051346), and Typhimurium_strain_LT2 (Accession: NZ_DAANHR000000000). One human clinical isolate of *Salmonella* belonging to serovar *S.* Infantis, obtained from the Caribbean Public Health Agency (CARPHA), Trinidad and Tobago, was included in our panel of isolates subjected to WGS. Other publicly available *Salmonella* strains were randomly selected, downloaded, and calculated using core genome alignment using the following criteria for each serovar: detected along the broiler production chain, originating from the Caribbean (Jamaica and Barbados), South America (Brazil), USA, Europe (Italy) and Asia (China) as well as the availability of the raw sequences on the NCBI database. It was important to include other publicly available strains in our study due to the worldwide trade of in poultry and products, and because of the limited phylogenetic studies focusing on *Salmonella* detected from the broiler industry in the region. Therefore, any comparison with international/regional strains was deemed valuable.

Core Genome Phylogeny was inferred by creating a core genome alignment with Roary (Page et al. 2015) and using the resulting alignment in IQ-TREE v.2.1.2 (Minh et al. 2020) to produce a tree file and 1,000 bootstrap pseudo-replications. The tree file was visualized with the R package ggtree (Yu et al. 2016). Bootstrap values ≥ 70% were considered well-supported/reliable and were displayed in the respective figures.

**Statistical analyses**

Chi-square test of independence analyses was conducted using the Statistical Product and Service Solutions, SPSS (version 27, IBM Corp., Somers, NY) to determine statistically significant associations in the frequency of isolation of *Salmonella* spp. The level of significance was set at an alpha level of 0.05.

**Results**

**Table S1** Frequency of isolation of *Salmonella* from samples in the batch sampling study

| **Study** | **Type of sample** | **Time of sample collection** | **No. samples collected** | **No. (%) positive for *Salmonella*** | **Serotypes (No., %)[a]** |
|---|---|---|---|---|---|
| Farm | Cloacal swab | Day 0<br>(10 days old) | 7 | 4 (57.1) | Infantis (4, 100.0) |
| Farm | Cloacal swab | 1 week pre-slaughter<br>(35 days old) | 25 | 12 (48.0)[a] | Infantis (11, 91.7) |
| | *Sub-Total* | | 32 | 16 (50.0) | |
| Processing plant | Pre-slaughter cloacal swabs | Day of slaughter<br>(39 days old) | 20 | 0 (0.0) | NA[b] |
| | Neck skins | | 10 | 5 (50.0)[a] | Infantis (3, 60.0) |
| | | | | | Kentucky (1, 20.0) |
| | Pre-packaged whole birds | | 5 | 1 (20.0) | Infantis (1, 100.0) |
| | Pre-packaged chicken parts | | 5 | 1 (20.0) | Infantis (1, 100.0) |
| | Sub-Total | | 40 | 7 (17.5) | |
| Retail outlet | Chilled chickens | | 10 | 4 (40.0) | Infantis (3, 75.0) |
| | | | | | Typhimurium (1, 25.0) |
| | *Total* | | 82 | 27 (32.9) | |
| *p value* | | | | 0.030 | |

[a] Traditional serotyping method conducted on 25 *Salmonella* isolates due to the high cost of traditional serotyping
[b] NA: Not available

**Table S2** Phenotypic frequency of resistance of *Salmonella* isolated during the batch sampling study

| | | | **No. (%) isolates resistant[b]:** | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Study** | **No. of isolated tested** | **No. (%) isolates resistant[a]** | **AMC** | **DO** | **CRO** | **CN** | **K** | **SXT** |
| Farm | 34 | 34 (100.0) | 3 (8.8) | 34 (100.0) | 34 (100.0) | 34 (100.0) | 34 (100.0) | 33 (97.1) |
| Processing plant | 10 | 10 (100.0) | 0 (0.0) | 10 (100.0) | 8 (80.0) | 9 (90.0) | 9 (90.0) | 8 (80.0) |
| Retail outlet | 5 | 5 (100.0) | 0 (0.0) | 5 (100.0) | 3 (60.0) | 3 (60.0) | 4 (80.0) | 3 (60.0) |
| Total | 49 | 49 (100.0) | 3 (6.1) | 49 (100.0) | 45 (91.8) | 46 (93.9) | 47 (95.9) | 44 (89.8) |
| *p value* | | | 0.494 | NA[c] | 0.003 | 0.002 | 0.061 | 0.02 |

[a] Resistance to one or more agents tested

[b] AMC:Amoxycillin-clavulanic acid, DO: Doxycycline; CRO: Ceftriaxone; CN: Gentamicin; K: Kanamycin; C: Chloramphenicol; SXT: Sulphamethoxazole- trimethoprim and CIP: Ciprofloxacin

[c] All the 49 isolates of *Salmonella* were susceptible to chloramphenicol and ciprofloxacin

**Table S3** Sources of serovar Infantis isolates (n = 21)

| Sample Identification | ST | Study type | Source | Type of sample | Year of isolation | Sampling level | SISTR serovar | Country source of isolate |
|---|---|---|---|---|---|---|---|---|
| Infantis_strain_119944 | 32 | Reference | Reference | Human faeces | 2008 | Reference | Infantis | Israel |
| SAMN25867770 | 32 | Longitudinal | Farm | Cloacal swab | 2019 | Farm | Infantis | Trinidad and Tobago |
| SAMN16678579 | 32 | Longitudinal | Farm | Cloacal swab | 2019 | Farm | Infantis | Trinidad and Tobago |
| SAMN25867771 | 32 | Longitudinal | Farm | Cloacal swab | 2019 | Farm | Infantis | Trinidad and Tobago |
| SAMN16678582 | 32 | Longitudinal | Processing Plant | Chilled whole carcass | 2019 | Processing Plant | Infantis | Trinidad and Tobago |
| SAMN16678577 | 32 | Longitudinal | Farm | Cloacal swab | 2019 | Farm | Infantis | Trinidad and Tobago |
| SAMN16678578 | 32 | Longitudinal | Farm | Cloacal swab | 2019 | Farm | Infantis | Trinidad and Tobago |
| SAMN16678576 | 32 | Longitudinal | Farm | Cloacal swab | 2019 | Farm | Infantis | Trinidad and Tobago |
| SAMN25867773 | 32 | Longitudinal | Supermarket | Chicken carcass | 2019 | Retail Outlet | Infantis | Trinidad and Tobago |
| SAMN16678583 | 32 | Longitudinal | Processing Plant | Chilled chicken parts | 2019 | Processing Plant | Infantis | Trinidad and Tobago |
| SAMN16678584 | 32 | Longitudinal | Supermarket | Chicken carcass | 2019 | Retail Outlet | Infantis | Trinidad and Tobago |
| SAMN16678586 | 32 | Longitudinal | Supermarket | Chicken carcass | 2019 | Retail Outlet | Infantis | Trinidad and Tobago |
| SAMN18407134 | 32 | Public Data | Processing Plant | Chilled whole carcass | 2021 | Processing Plant | Infantis | USA |
| SAMN15964920 | 32 | Public Data | Supermarket | Chicken carcass | 2019 | Retail Outlet | Infantis | Barbados |
| SAMN15964921 | 32 | Public Data | Supermarket | Chicken carcass | 2019 | Retail Outlet | Infantis | Barbados |
| SAMN15964932 | 32 | Public Data | Supermarket | Chicken carcass | 2019 | Retail Outlet | Infantis | Barbados |
| SAMEA3538994 | 32 | Public Data | Processing Plant | Chilled whole carcass | 2007 | Processing Plant | Infantis | Italy |
| SAMEA3539001 | 32 | Public Data | Processing Plant | Chilled whole carcass | 2014 | Processing Plant | Infantis | Italy |
| SAMN08951144 | 32 | Public Data | Processing Plant | Chilled whole carcass | 2016 | Processing Plant | Infantis | Brazil |
| SAMN14404289 | 32 | Control | Human clinical | Human faeces | 2019 | Control | Infantis | Trinidad and Tobago |
| SAMN16986393 | 32 | Public Data | Pluck Shop | Chicken carcass | 2014 | Retail Outlet | Infantis | China |

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