Genomic Comparison of Eight Closed Genomes of Multidrug-Resistant *Salmonella enterica* Strains Isolated From Broiler Farms and Processing Plants in Trinidad and Tobago

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Salmonella enterica is an important foodborne pathogen worldwide. We used long and short-read sequencing to close genomes of eight multidrug-resistant (MDR) *S. enterica* strains, belonging to serovars Infantis (2), Albany, Oranienburg, I 4,[5],12:i:-, Javiana, Schwarzenburg, and Kentucky from broiler chicken farms and processing plants in Trinidad and Tobago. They also belonged to seven different sequence types (STs-32, 292, 1510, 19, 24, 152, and 96). Among the strains, seven had demonstrated multi-drug resistance with the presence of at least three AMR genes, whereas three isolates contained the quinolone resistance gene *qnr*<sub>B19</sub> in plasmids (CFSAN103840, CFSAN103854, and CFSAN103872). The extended-spectrum β-lactamase genes *bla<sub>CTX-M-65</sub>* (CFSAN103796) and *bla<sub>TEM-1</sub>* (CFSAN103852) were detected in this study. The genomes closed in this study will be useful for future source tracking and outbreak investigations in Trinidad and Tobago and worldwide.

**Keywords:** Salmonella, multi-drug resistance, complete genome, nanopore sequencing, poultry, Trinidad and Tobago

**SIGNIFICANCE**

*Salmonella enterica* contamination of poultry is a major route of transmission to humans. We have closed genomes of multiple *Salmonella* serovars and report multiple antimicrobial resistance (AMR) genes from isolates collected in processing plants and retail markets in Trinidad and Tobago. These closed genomes will aid in monitoring AMR transmission and future outbreak investigations.

**INTRODUCTION**

*Salmonella enterica* is one of the most important foodborne pathogens in the world (Gonzalez-Escalona et al., 2009). There are more than 325,000 *Salmonella* genomes in NCBI encompassing...
isolates from many countries (https://www.ncbi.nlm.nih.gov/pathogens/). Non-typhoidal Salmonella (NTS) is responsible for ~93,750,000 cases of gastroenteritis and 155,000 deaths worldwide each year. Nearly 86% are linked to foodborne transmission (Majowicz et al., 2010). Poultry, including eggs and egg products, is one of the main reservoirs of Salmonella and has often been implicated as the source of many outbreaks worldwide (Schoeni et al., 1995; Kuehn, 2010; Jackson et al., 2013; Deng et al., 2014; Antunes et al., 2016).

Salmonella serovars Enteritidis, Newport, Typhimurium, Javiana, I 4,[5],12:i:-, and Heidelberg have been among the top five serovars associated with human illness over the last 15 years (2006–2016) in the United States (https://www.cdc.gov/nationalsurveillance/pdfs/2016-Salmonella-report-508.pdf). S. serovar Infantis has shown the highest percent increase in reported cases over the same time frame (+168.5%). While, S. serovar Kentucky has become the most commonly detected serovar in chicken (Foley et al., 2011), but is not among the top 20 serovars causing human illness.

While most Salmonella infections result in gastroenteritis without the need for additional medical intervention, severe, invasive infections can require antimicrobial treatment. Multidrug resistance (MDR) in Salmonella is closely monitored in clinical and environmental isolates around the world [National Antimicrobial Resistance Monitoring System (NARMS), European Center for Disease Control and Prevention (ECDC), and European Food Safety Authority (EFSA)]. In the United States, the FDA has issued guidance on the judicious use of antimicrobial agents in food-producing animals (http://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-209-judicious-use-medically-important-antimicrobial-drugs-food-producing-animals) and individual antimicrobial agent restrictions have resulted in the decreased prevalence of resistance-conferring genes (https://www.fda.gov/media/108304/download).

Several antimicrobial-resistant (AMR) Salmonella strains were isolated during a Salmonella and AMR surveillance program of poultry production facilities in Trinidad and Tobago from 2016 to 2019 (Khan et al., 2022b). Their genomes were sequenced by short-read sequencing, and we selected a group of eight strains belonging to multiple serovars and showing a diverse range of MDR, to completely close their genomes using a combination of long-read and short-read sequencing technologies. The availability of these closed genomes will be useful for future outbreak investigations and for understanding AMR acquisition and transmission in Salmonella within the local Trinidad and Tobago region and worldwide.

MATERIALS AND METHODS
Bacterial Strains and Media
Cloacal swabs, drag swabs, whole chicken carcasses, and neck skin samples were collected from retail outlets, processing plants, and farms in Trinidad and Tobago during an AMR surveillance program from 2016 to 2019 (Khan et al., 2022b). The sample was pre-enriched in buffered peptone water (Oxoid, Ltd., Hampshire, UK) for 18–24 h at 37°C, then selectively enriched in tetrathionate broth (Oxoid), and thereafter incubated for 18–24 h at 37°C. The sample enriched in selective broth was then sub-cultured onto xylose lysine Tergitol 4 (Oxoid) and incubated aerobically at 37°C for 18–24 h. Suspected Salmonella colonies that displayed characteristic colonies on the selective agar plate were then purified on blood agar plates (Oxoid) and incubated at 37°C for 18–24 h. Pure cultures were subjected to a panel of biochemical tests that included triple sugar iron agar, lysine iron agar, urea, citrate, methyl red, sulfide-indole-motility medium, and o-nitrophenyl-b-D-galactopyranoside (Oxoid) (Andrews, 1992). Biochemically confirmed isolates were then subjected to serological typing by using Salmonella polyvalent antisera (A-I and Vi, Difco, Detroit, MI). Complete confirmation and serotyping of Salmonella isolates were performed by using the phase reversal technique, and the results were interpreted according to the Kauffmann-White scheme (https://www.pasteur.fr/sites/default/files/veng_0.pdf) at the Public Health Laboratory, Ministry of Health, St. Michael, Barbados. The single isolate strains were grown overnight in tryptic soy broth (TSB) medium at 35°C. The strains to have their genomes entirely closed were selected among 146 strains isolated by Khan et al. (2022b). The criteria employed for their selection was: (1) a representative of each predominant serovar from that study [Kentucky 20.5% (30/146), Javiana 19.2% (28/146), Infantis 13.7% (20/146), and Albany 8.9% (13/146)], (2) Infantis with a different AMR profile, and 3) uncommon serotypes isolated from poultry [Oranienburg, 14,[5],12:i:- (a monovarant of Salmonella Typhimurium), and Schwarzengrund].

Serotyping and Antimicrobial Resistance Determination
Conventional serotyping methods using the phase reversal technique described previously (Khan et al., 2021, 2022a) were performed at the Public Health Laboratory, Ministry of Health, St. Michael, Barbados. The antimicrobial resistance was determined by the disk diffusion method as described previously (Khan et al., 2021, 2022a). Data generated from these two methods were compared to the genomic data.

Nucleic Acid Extraction
DNA was extracted using the Maxwell RSC cultured cells DNA kit with a Maxwell RSC instrument (Promega, Madison, WI, USA) following the manufacturer’s protocols for Gram-negative bacteria with additional RNase treatment. DNA concentration was determined by the Qubit 4 Fluorometer (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions.

Illumina MiSeq Short-Read Sequencing
The short reads whole-genome sequence for this strain was generated by Illumina MiSeq sequencing with the MiSeq V3 kit using 2 × 250 bp paired-end chemistry (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. The libraries were constructed using 100 ng of genomic DNA using the Illumina® DNA Prep (M) Tagmentation (Illumina), according
to the manufacturer’s instructions. Reads were trimmed with Trimmomatic version 0.36 (Bolger et al., 2014).

**Oxford Nanopore Long-Read Sequencing**

The long reads for each strain were generated through GridION sequencing (Nanopore, Oxford, UK). The sequencing library was prepared using the rapid barcoding sequencing kit (SQK-RBK004). Each library was run in an FLO-MIN106 (R9.4.1) flow cell, according to the manufacturer’s instructions, for 48 h. Default parameters were used for all software unless otherwise specified. The run was base called live with default settings (MinKNOW Core version 3.6.5, Bream version 4.3.16, and Guppy version 3.2.10). Reads <4000 bp and quality scores of <7 were discarded for downstream analysis, for an estimated genome average coverage of 59 – 250X.

**Contig Assembly and Annotation**

The final complete genome [chromosome and plasmid(s), when present] for each strain was obtained using a previously described pipeline (Gonzalez-Escalona et al., 2018) with modifications. The final genomes were achieved by a hybrid assembly generated using both nanopore and MiSeq data with Unicycler version 4.8 (Wick et al., 2017). A second de novo genome assembly was obtained with Flye version 2.8 (Kolmogorov et al., 2019) using nanopore data. Both assemblies (Flye and Unicycler) for each strain were aligned with Mauve version 2.4 (Darling et al., 2004). If the two aligned assemblies agreed in synteny and size, then the Unicycler hybrid assembly was used as the final assembly (i.e., complete genome). Unicycler assembled the chromosomes and plasmids as circular closed and oriented the chromosomes to start at the dnaA gene. The genomes were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP, http://www.ncbi.nlm.nih.gov/genome/annotation_prok) (Tatusova et al., 2016). In silico analysis was used to determine the multi-locus sequence type (MLST) with the MLST website for *Salmonella* (http://enterobase.warwick.ac.uk/species/index/senterica), serotype using SeqSero (Zhang et al., 2015) (http://www.denglab.info/Seqero) which infers the serovar from antigenic structure, and antimicrobial genes were found using ResFinder-4.1 (https://cge.cbs.dtu.dk/services/ResFinder/) (Bortolaia et al., 2020). In silico virulence profile was determined using the software Ridom Seqsphere+ version 6.0.2 (Ridom GmbH, Germany) and their task tool that uses the virulence factor database (VFDB) as a reference (Liu et al., 2019) to assign virulence factors.

**Genomic Data Analysis**

To analyze the relationships among the eight NTS closed genomes we performed a core genome MLST (cgMLST) using Ridom Seqsphere+ version 6.0.2 (Ridom) as previously described (Toro et al., 2016). The genome of *S. Enteritidis* P125109 (NC_011294) was used as a reference genome (4,160 genes) (Thomson et al., 2008). The *S. Enteritidis* genome from strain EC20121175 (CP007269.2) was used for comparison with the reference genome to establish a list of core and accessory genome genes. Genes that are repeated in more than one copy in any of the two genomes were removed from the analysis as failed genes. A task template was created that contains both core and accessory genes for this reference SE strain for any future testing. Each individual locus (core or accessory genes) from strain P125109 was assigned allele number 1. The assemblies for each individual NTS closed genome in this study were queried against the task template and if the locus was found and was different from the reference genome or any other queried genome already in the database, a new number was assigned to that locus, and so on. After eliminating any loci that were missing from the genome of any strain used in our analyses, we performed the cgMLST analysis. These remaining loci were considered the core genome shared by the analyzed strains. We used Nei’s DNA distance method (Nei et al., 1983) for calculating the matrix of genetic distance, taking only the number of same/different alleles in the core genes into consideration. A neighbor-joining (NJ) tree using the appropriate genetic distances was built after the cgMLST analysis.

**Plasmid Comparison**

A comparison of the CFSAN103796 *blaCTX-M–65* -positive plasmid with the ones reported in Tate et al. (2017) was performed and visualized with CGView (https://proksee.ca/) (Stothard and Wishart, 2005).

**Data Deposition**

The complete genome sequences of the eight *S. enterica* strains have been deposited at GenBank under the accession listed in Table 1.

**RESULTS AND DISCUSSION**

An AMR surveillance program of poultry farms and retail outlets in Trinidad and Tobago from 2016 to 2019 produced several *Salmonella* isolates that were confirmed to be *S. enterica* by selective microbiological plating. Initial sequencing efforts identified the sequence type (ST) and serovar for each sample by in silico analysis. We selected eight samples from multiple serovars to completely close genomes using a hybrid assembly with Illumina MiSeq short-read and Oxford Nanopore long-read sequencing. The short-read sequencing for these eight samples was performed over three sequencing runs with an average output of 12.6 Gb and estimated average genome coverage of 50–150×. The multiplexed nanopore sequencing for eight samples was carried out over two sequencing runs. The output for run 1 was 7.69 Gb (2.33 million reads with a quality score of 9.48 and N50=11,835 bp) and for running 2 was 3.71 Gb (694 thousand reads with a quality score of 9.93 and N50=12,930 bp). The estimated average genome coverage was 59–250× (Table 1).

The final closed genomes were generated by a hybrid assembly (Unicycler), which matched in synteny and size to a de novo assembly generated using only nanopore reads by the Flye assembler. In silico analysis confirmed the ST and serovar for each closed genome observed with the
TABLE 1 | Metadata for the eight Salmonella enterica strains isolated from chicken on poultry farms and processing plants were reported in this study.

| CFSAN No. | Accession No. chromosome and plasmid(s) (size bp) | GC content (%) | Hybrid assembly genome coverage (X) | Illumina SRAs (Read no.) | Nanopore SRAs (Read no.) | AMR genes | ST | Serotype |
|-----------|--------------------------------------------------|----------------|-------------------------------------|-------------------------|-------------------------|------------|----|----------|
| CFSAN103796 | CP066335 (4,727,835) | 52.3 | 95 | SRR11660184 (2,096,092) | SRR13073499 (47,009) | mdsA, mdsB, mdsC, aac(6')-Iaa | 32 | Infantis |
| | CP066336 (312,952) | 50.3 | 83 | | | tetA, aph(3'), suF1, aacA1, aac(3)-Iva, aph(4)-la, blaCTX-M-65, dfrA14, qacEdelta1 | |
| CFSAN103854 | CP066333 (4,944,981) | 52.2 | 133 | SRR11660185 (2,482,592) | SRR13073498 (61,306) | mdsA, mdsB, mdsC, aac(6')-Iaa | 292 | Albany |
| | CP066334 (3,071) | 48.2 | 33 | | | | |
| CFSAN103872 | CP066260 (5,053,975) | 52.2 | 80 | SRR11660187 (1,533,662) | SRR13073497 (41,128) | mdsA, mdsB, mdsC, aac(6')-Iaa | gnrB19 | |
| | CP066261 (49,044) | 49.3 | 265 | | | | |
| | CP066262 (5,320) | 52.4 | 400 | | | | |
| CFSAN103852 | CP066328 (4,836,001) | 52.2 | 129 | SRR129965791 (2,606,746) | SRR13073496 (70,597) | mdsA, mdsB, mdsC, aac(6')-Iaa | 1510 | Oranienburg |
| | CP066329 (110,489) | 50.2 | 189 | | | | |
| | CP066330 (93,844) | 53.1 | 228 | | | | |
| | CP066331 (33,659) | 41.3 | 310 | | | | |
| | CP066332 (1,749) | 57.5 | 1400 | | | | |
| CFSAN103840 | CP066325 (4,554,367) | 52.1 | 105 | SRR12996802 (2,110,046) | SRR13073495 (63,239) | mdsA, mdsB, mdsC, aac(6')-Iaa | 24 | Javiana |
| | CP066326 (2,579) | 50.5 | 1000 | | | | gnrB19 |
| | CP066327 (1,395) | 55.2 | 3000 | | | | |
| CFSAN103816 | CP066324 (4,703,182) | 52.3 | 59 | SRR12996817 (2,060,084) | SRR13073494 (53,365) | mdsA, mdsB, mdsC, aac(6')-Iaa | 32 | Infantis |
| | CP066321 (4,847,947) | 52.2 | 125 | SRR12996807 (1,866,844) | SRR13073493 (106,256) | mdsA, mdsB, mdsC, aac(6')-Iaa | 96 | Schwarzengrund |
| | CP066322 (144,839) | 49.0 | 198 | | | | |
| | CP066323 (4,050) | 42.8 | 700 | | | | |
| CFSAN103828 | CP066318 (4,767,999) | 52.2 | 30 | SRR12996770 (1,746,768) | SRR13073492 (50,574) | mdsA, mdsB, mdsC, aac(6')-Iaa | 152 | Kentucky |
| | CP066319 (146,829) | 49.4 | 48 | | | | |
| | CP066320 (46,120) | 42.8 | 150 | | | | |

*a* In silico antimicrobial genes were found using ResFinder-4.1 (https://cge.cbs.dtu.dk/services/ResFinder/).

*b* In silico MLST used the MLST website for Salmonella (http://enterobase.warwick.ac.uk/species/index/senterica).

*c* In silico serotyping using SeqSero (Zhang et al., 2015) (http://www.denglab.info/SeqSero), a tool to infer serovar from the genes that determine the antigenic structure.

*d* Othmarschen by phenotypic method.
preliminary analysis run using the short-read sequencing (Khan et al., 2022b) (Table 1). The strains represented seven different STs and serotypes (32—Infantis, 292—Albany, 1,510—Oranienburg, 19−1,4,[5],12:i:- (Typhimurium), 24—Javiana, 96—Schwarzengrund and 152—Kentucky). There was an 88% agreement (7/8) in the serotyping results by phenotypic and in silico WGS (SeqSero) methods (Table 1). Strain CFSAN103872 was identified as Othmarschen by the phenotypic method but as Oranienburg by SeqSero. This discrepancy can be explained by the fact that both serotypes have the same antigenic formula (6,7:m,t-) and other authors showed that they were undisguisable genetically (Robertson et al., 2018). The genome size of these NTS strains varied from 4.6 Mb (Javiana, CFSAN103840) to 5.1 Mb (Oranienburg, CFSAN103872), highlighting the plasticity of NTS genomes (Table 1). A cgMLST analysis showed that these strains were highly divergent but shared at least 3,432 genes out of a total of 4,160 genes of the reference S. Enteritidis P125109 (NC_011294) strain used for this cgMLST analysis (Figure 1A; Supplementary Table 1). A minimum spanning tree of the cgMLST analysis showed that they differed in most loci, >78% (Figure 1B). The two Infantis (ST32) differed in at least 136 loci, which was expected since Infantis strain CFSAN103816 lacked the MDR plasmid, indicating that these two strains belonged to very different clones circulating in the same country at the same time.

The closed genomes were further analyzed for the presence of AMR genes using the ResFinder database (Table 1) (Bortolaia et al., 2020). All strains were found to contain mdsA, mdsB, and mdsC components of the MdsABC complex in the chromosome. MdsABC confers resistance to multiple toxic compounds, including novobiocin, acriflavine, crystal violet, ethidium bromide, methylene blue, rhodamine 6G, tetraphenylphosphonium bromide, benzalkonium bromide, and SDS and oxidative stress-inducing compounds (diamide, H2O2, Paraquat), and is necessary for colonization of host cells (Nishino et al., 2006; Song et al., 2015). Seven of the strains

| Strain ID | Plasmid size (bp) | Plasmid type | Genes | GenBank accession No. |
|-----------|------------------|--------------|-------|-----------------------|
| CFSAN103796 | 312,952 | IncFIB | aph(4)-ia, aph(4)-Ia, aac(3)-Iva, blaCTX-M-65, dfrA14 | tetA, sul1, aadA1, qacEdelta1 | CP066336 |
| 2014AM-3028 | 316,160 | IncFIB | aph(4)-ia, aac(3)-Iva, blaCTX-M-65, floR, dfrA14 | sul1, tetA, aadA1 | CP016411 |
| N55391 | 316,814 | IncFIB | aph(4)-ia, aph(3′)-Ic, aac(3)-Iva, blaCTX-M-65, floR, dfrA14 | sul1, tetA | CP016411 |
| FSIS1502169 | 323,122 | IncFIB | aph(4)-ia, aph(3′)-Ic, aac(3)-Iva, floR, dfrA14 | sul1, tetA, aadA1 | CP016407 |
| FSIS1502916 | 322,518 | IncFIB | aph(4)-ia, aph(3′)-Ic, aac(3)-Iva, floR, dfrA14 | sul1, tetA, aadA1 | CP016409 |
demonstrated multi-drug resistance with the presence of at least three AMR genes. Aminoglycoside, tetracycline, and sulfonamide resistance genes were detected in seven strains: CFSAN103796 [aac(6′), tetA, aph(3′)], sul1, aadA1, aac(3)-Iva, aph(4)-Ia], CFSAN103854 [aac(6′)-Iaa], CFSAN103872 [aac(6′)-Iaa, aph(3′)-Ib, aph(6)-Id],...
CFSAN103852 [\textit{aac(6')-Iaa}, \textit{aph(3'')-lb}, \textit{aph(6'}-ld)], CFSAN103816 [\textit{aac(6')-Iaa}], CFSAN103862 [\textit{aac(6')-Iaa}, \textit{aph(3'')-lb}, \textit{aph(6}-ld]}, and CFSAN103828 [\textit{aac(6')-Iaa}, \textit{aph(3'')-lb}, \textit{aph(6}-ld, \textit{tetB}] (Table 1).

In clinical \textit{Salmonella} isolates, combined resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT) decreased from 8.2% in 1996 to 3.6% in 2009 (Medalla et al., 2013), but other medically relevant antibiotics have become a recent focus of study. Synthetically derived fluoroquinolones were introduced to combat ACSSuT resistance. Three isolates contained the quinolone resistance gene \textit{qnrB19} in plasmids (CFSAN103840, CFSAN103854, and CFSAN103872) of different sizes. Plasmid-mediated quinolone resistance was not identified in retail meat products until 2017 (Sjölund-Karlsson et al., 2010; Tyson et al., 2017). The presence of these small plasmids carrying the \textit{qnrB19} gene is worrisome since they have been identified in other countries in Latin America, such as Peru, Bolivia, Colombia, and Argentina, and appear to be highly mobile among different microorganisms (Tran et al., 2012).

The \(\beta\)-lactams, including ampicillin and cephalosporin, are another treatment for invasive salmonellosis, particularly in children. Ceftriaxone resistance increased in the US from 10% in 2002 to 38% in 2010 in chickens, prompting the prohibition of cephalosporin by the FDA in 2012. The extended-spectrum \(\beta\)-lactamase genes \textit{bla}_{CTX-M-65} (CFSAN103796) and \textit{bla}_{TEM-1} (CFSAN103852) were detected in this study on plasmid pCFSAN103796 (312,952 bp) and on the chromosome, respectively. An alignment of pCFSAN103796 against several \textit{bla}_{CTX-M-65}–containing plasmids reported by Tate et al. (2017), showed pCFSAN103796 was highly similar but differed, besides size and SNPs, in two of the main AMR sites from the other reported plasmids (Table 2; Figure 2). There were at least two genes missing in Site 1 that were present in the other plasmids.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{cgMLST analysis of six \textit{S. Infantis} strains showing the divergent evolution of CFSAN103796 from the US strains (2014AM-3028, N55391, FSIS1502169, and FSIS1502916) and the other \textit{S. Infantis} closed genome from Trinidad and Tobago (CFSAN103816). CFSAN103816 genome was used as an outgroup based on 3768 shared genes. \textbf{(A)} NJ tree showing the genetic differences among these genomes based on the shared loci rooted in CFSAN103816. \textbf{(B)} Minimum spanning tree allowing to visualize the number of loci differences between these genomes. Numbers by lines represent the number of loci differing between strains. The two \textit{S. Infantis} from Trinidad and Tobago are shown in the colored boxes. The lines are not drawn to scale.}
\end{figure}
TABLE 3 | Phenotypic and plasmid classification per strain from this study.

| CFSAN No.          | Phenotypic AST test results (sensitivity)* | Plasmid type (plasmid accession No.)* | Virulence factors‡ |
|--------------------|-------------------------------------------|---------------------------------------|--------------------|
| CFSAN103796        | P (S), TE (R), CE (R), AM (R), PH (S), S (R), F (S) | IncFIB (CP066336) | csg, bcf, lpf, TTSS (SPI-1 encode), TTSS (SPI-2 encode), TTSS-1 translocated effectors |
| CFSAN103854        | P (S), TE (R), CE (S), AM (R), PH (S), S (R), F (S) | Col-pHAD28 (CP066334) | csg, bcf, lpf, TTSS (SPI-1 encode), TTSS (SPI-2 encode), TTSS-1 translocated effectors |
| CFSAN103872        | P (S), TE (R), CE (R), AM (R), PH (S), S (R), F (S) | IncN2 (CP066261) | csg, bcf, lpf, TTSS (SPI-1 encode), TTSS (SPI-2 encode), TTSS-1 translocated effectors |
| CFSAN103852        | P (R), TE (R), CE (R), AM (R), PH (S), S (R), F (S) | IncI-I (CP066329), IncFIB/IncFil (CP066330), IncX1 (CP066331), ColpVC (CP066332) | gogB, grvA, csg, bcf, lpf, TTSS (SPI-1 encode), TTSS (SPI-2 encode), TTSS-1 translocated effectors |
| CFSAN103840        | P (S), TE (R), CE (R), AM (R), PH (S), S (R), F (S) | Col-pHAD28 (CP066326) | csg, bcf, TTSS (SPI-1 encode), TTSS (SPI-2 encode), TTSS-1 translocated effectors |
| CFSAN103816        | P (S), TE (S), CE (R), AM (R), PH (S), S (R), F (S) | NA | csg, bcf, TTSS (SPI-1 encode), TTSS (SPI-2 encode), TTSS-1 translocated effectors |
| CFSAN103862        | P (S), TE (R), CE (R), AM (R), PH (S), S (R), F (S) | IncFIB/IncFIC (CP066322) | csg, bcf, TTSS (SPI-1 encode), TTSS (SPI-2 encode), TTSS-1 translocated effectors |
| CFSAN103828        | P (S), TE (R), CE (R), AM (R), PH (S), S (R), F (S) | IncFIB/IncFII (CP066319), IncX1 (CP066320) | csg, bcf, TTSS (SPI-1 encode), TTSS (SPI-2 encode), TTSS-1 translocated effectors |

*P, penam (amoxicillin–clavulanic acid, 30 µg); TE, tetracycline (doxycycline, 30 µg); CE, cephalosporin (ceftaxone, 30 µg); AM, aminoglycoside (gentamicin, 10 µg, and kanamycin, 30 µg); PH, phenicol (chloramphenicol, 30 µg); S, sulfonamide (sulfamethoxazole–trimethoprim, 23.75 and 1.25 µg); F, fluoroquinolone (ciprofloxacin, 5 µg).

‡Incompatibility group replicon.

*For the complete list, please go to Supplementary Table 2.

(floR, and fosA3) while pCFSAN103796 has acquired a qacEdelta1 gene on Site 2 (Table 2).

Core genome multi-locus sequence type analysis of these 5 Infantis strains (Table 2) and CFSAN103816 (as an outgroup) showed that the 4 strains differed from CFSAN103796 by a minimum of 19 loci differences, further confirming the divergent evolution of this strain from the other ones isolated in the US (Figure 3). Furthermore, Brown et al. (2018) described the presence of \( \text{bla}_{CTX-M-65} \)-containing Salmonella enterica serotype Infantis isolates in the US that were grouped in the same phylogenetic clade as MDR Peruvian isolates (Brown et al., 2018), illustrating the intercontinental spread of this AMR gene. This latter fact highlights the importance of closing the genomes of strains carrying this important AMR gene to track the spreading and evolution of this plasmid through gene acquisitions and SNP accumulations during all these years and in different regions of the world. In turn, this type of analysis could allow establishing the route of dissemination around the world for this strain/plasmid combination as has been done for S. Enteritidis (Li et al., 2021). We have additionally detected the presence of the \( \text{mcr-9} \) gene in another strain isolated from this overall study (Maguire et al., 2021). The \( \text{mcr-9} \) gene variant was described in 2019 (Carroll et al., 2019) and appears not to confer colistin resistance in Salmonella and Escherichia coli (Tyson et al., 2020).

Further analysis of the presence or absence of reported virulence genes for S. enterica was determined by an in silico approach using the data available in the virulence factor database (VFDB) for that bacterium (Liu et al., 2019) (Table 3). As reported in that table (the complete virulence gene repertoire is available in Supplementary Table 2), all closed Salmonella genomes from this study carried both Salmonella pathogenicity islands SPI-1 and SPI-2, as reported previously by others (Hoffmann et al., 2016; Lou et al., 2019). SPI-1 contains genes encoding various components of a type III secretion system (T3SS), its regulators, and its secreted effectors (Marcus et al., 2000). SPI-1 enables S. typhimurium to efficiently penetrate the intestinal epithelium and its role may be limited to the colonization of the gut (Marcus et al., 2000). While SPI-2 contains a second T3SS that is essential for survival within the macrophage and for the establishment of systemic infection (Kombade, 2021). The type III secretion system of SPI-2 is structurally and functionally distinct from that of SPI-1. These two SPIs are necessary for Salmonella intestinal epithelial invasion and virulence. An interesting finding was that strain CFSAN103862 serotype Schwarzengrund lacked \( \text{pggH}, \ -I, \ -J, \ -K \) and \( \text{orgA} \) genes in the SPI-1. These genes are required for the secretion of invasion effector proteins and mutant strains lacking these genes were attenuated for invasion in a standard invasion assay of HEp-2 cell monolayers (Fahlen et al., 2000). Some did not carry the \( \text{lpf} \) operon (CFSAN103840—Javiana, and CFSAN103862—Schwarzengrund) which has been shown to be crucial for the attachment of Salmonella enterica serovar Typhimurium to murine Peyers’s patches (Bäumler et al., 1996). The lack of these many genes in this particular Schwarzengrund strain suggests that it might be less pathogenic to humans since invasion and attachment might be impaired. However proper experiments must be conducted in the future to prove this theory.

Strain CFSAN103852, a monophasic variant of S. Typhimurium was the only one carrying both gogB and grvA genes. gogB, an anti-inflammatory effector, helps Salmonella regulate inflammation-enhanced colonization by limiting tissue damage during infection (Pilar et al., 2012). While grvA, an anti-virulence gene located on the Gifsy-2 phage of S. Typhimurium, participates actively...
in modulating its pathogenicity (Ho and Slauch, 2001). The presence of these genes only in this monophasic S. Typhimurium confirms that the pathogenicity of different Salmonella serotypes is a complex system and might include redundant systems. Most of these eight strains appear to be pathogenic to humans, although with some differences in virulence profiles.

We further reported the plasmid type and the phenotypic AST test results for the eight strains sequenced in this study. The plasmids carried by these strains contained at least six different incompatibility group replicons, with four of the strains carrying plasmids with an IncFIB incompatibility group replicon (Table 3), which has been reported in many MDR plasmids (Tate et al., 2017).

CONCLUSION

The presence of MDR NTS in poultry environments remains a significant global challenge for public health. With the increasing reports of newer variants of AMR genes that confer resistance to diverse types of antibiotics, the need to enact active AMR gene surveillance studies is paramount. With these types of studies, we can not only detect the presence and spread of important AMR genes in poultry, but also identify the strains carrying these AMR genes and their location (whether in the chromosome or plasmids). Here we closed eight genomes of several MDR NTS, determine their ST, serotype, and associated AMR gene profiles, which will be useful for future source tracking, and outbreak investigations in Trinidad and Tobago and worldwide.

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DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

MM and NG-E analyzed the data and wrote the manuscript. MM performed laboratory experiments. MM, AK, AA, KG, and NG-E provided materials and critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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