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

Anisa S. Khan[1], Rian Ewald Pierneef[2], Woubit Abebe[3], Abiodun A. Adesiyun [1,4*]

[1]School of Veterinary Medicine, Faculty of Medical Sciences, University of the West Indies, St. Augustine, Trinidad and Tobago; anisakhan11@gmail.com (ORCID 0000-0002-6855-1410)

[2]Agricultural Research Council-Biotechnology Platform, 100 Old Soutpan Road, Onderstepoort, Pretoria 0110, South Africa; pierneefr@arc.agric.za (ORCID 0000-0002-0619-0058)

[3]Department of Pathobiology, Center for Food Animal Health, Food Safety and Food Defense, Tuskegee University, College of Veterinary Medicine, Tuskegee, 201 Frederick D Patterson Dr, Tuskegee, AL 36088, U.S.A.; wabdela@tuskegee.edu

[4]Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, Pretoria 0110, South Africa; abiodun.adesiyun@sta.uwi.edu (ORCID 0000-0001-9470-9421)

*Corresponding author: Abiodun A. Adesiyun, e-mail: Abiodun.adesiyun@sta.uwi.edu

Abstract

This study was conducted within 2 months by sampling chicken batches from a broiler farm, a plant processing plant, and supermarket retail. The overall frequency of isolation of *Salmonella* was 50% (16/32), 17.5% (7/40), and 40% (4/10) for the samples collected from the broiler farm, processing plant, and retail outlet, respectively. Serovar Infantis was the predominantly isolated serovar at the 3 sampling sites. Resistance genes *aac(3)IV*, *aph(4)Ia*, $bla_{CTX\text{-}M65}$, and *qacEdelta1* were detected in 84.6% (11/13) of the isolates subjected to whole genome sequencing. *S.* Infantis strains were clustered within and across the 3 sampling sites. This study demonstrates a direct measure of the transmission dynamics of *Salmonella* during a farm-to-fork approach.

Keywords: Antimicrobial resistance; Broilers; MDR; Longitudinal study; Phylogeny; Trinidad

1 Introduction

Worldwide, contaminated poultry and its products are significant causes for human salmonellosis (Cosby et al. 2015). Many studies conducted on the poultry production system in Trinidad and Tobago were cross-sectional in design, mostly with long intervening periods (Khan et al. 2018, 2021). These approaches make it difficult to assess the direct impacts of the findings on the farm-to-fork approach and food safety. Therefore, a 2-month study was conducted. By using a batch sampling method, chicken processing was followed from the broiler farm via slaughterhouse and processing plant, and finally, to the supermarket where the chicken carcasses were sold. This approach aimed to investigate the transmission of *Salmonella enterica* within a vertically integrated broiler production system during a standard broiler

production grow-out. Whole genome sequencing (WGS) was used to characterize *Salmonella* and determine the relatedness of the serovars of *Salmonella* isolated at 3 chicken production levels in Trinidad and Tobago.

2 Results and discussion

Of 4 farms sampled for the study, 1 had *Salmonella*-positive chickens and therefore, was chosen for this study. 4 (57.1%) of 7 and 12 (48%) of 25 cloacal swab samples collected from the poultry farm were positive for *Salmonella* on day 0 (10 days old) and day 35 (1-week pre-slaughter), respectively (Supplementary Material, Fig. S1). Within 2 months of sampling, *Salmonella* was detected in 50% (16/32), 17.5% (7/40), and 40% (4/10) of samples originating from one broiler farm, processing plant, and supermarket, respectively ($p = 0.03$). *S.* Infantis was isolated at all 3 production levels, whereas *S*. Kentucky was isolated only at broiler processing and *S.* Typhimurium at the retail outlet.

This is the first documented WGS study conducted in Trinidad and Tobago that directly followed a batch of chickens from a *Salmonella*-positive flock during the grow-out stages through processing at a plant and then to a retail outlet. It highlights the transmission throughout the production, processing, and retail chain and inadequate sanitization and disinfection protocols employed at the processing plants. This finding agrees with the serovars detected in our cross-sectional study conducted on 27 broiler farms (Khan et al. 2022a), where serovar Infantis was the most frequently isolated and accounted for 62.5% of all serovars found. *S.* Kentucky and Typhimurium could have originated from the farm or processing plant due to intermittent shedding at the farm or as a result of cross-contamination at the processing plant since chickens originating from other farms were processed before the tagged positive flock.

All 49 (100%) isolates of *Salmonella* from farms, processing plants, and retail outlets were resistant to one or more antimicrobial agents (Supplementary Material, Table S2). All isolates were susceptible to chloramphenicol and ciprofloxacin. All of the 34 farm isolates of *Salmonella* were resistant to doxycycline (DO), ceftriaxone (CRO), gentamicin (CN), and kanamycin (K). All isolates originating from the processing plant and retail outlet were resistant to doxycycline (DO).

The overall frequency of resistance exhibited by the *Salmonella* serovars was high (89.9% to 100%) to DO, CRO, CN, K, and trimethoprim/sulfamethoxazole (SXT). Similar resistances were detected in the isolates of *Salmonella* recovered from a cross-sectional study earlier conducted at hatcheries and broiler farms (Khan et al. 2022a). These findings can be attributed in part to the exposure of broilers on the farms to antimicrobial agents used for prophylaxis, therapy, and growth promotion since these agents are regulated but not routinely enforced (Khan et al. 2022a).

Resistance genes *aac(3)IV*, *aph(4)Ia*, $bla_{CTX\text{-}M65}$, and *qacEdelta1* were detected in 84.6% (11/13) of the isolates. All isolates except *S*. Kentucky ($n = 1$) contained resistance genes. The genotypic resistance profiles of the serovars detected throughout this study are shown in Table 1. Pattern A (farm, processing plant, and retail outlet) observed in serovar Infantis isolates was identical (*aac(3)-IV-aph(3')-Ia-aph(4)-Ia-qacEdelta1-*$bla_{CTX\text{-}M65}$*-sul1*). Pattern B (farm and processing plant) was identical (*aac(3)-IV-aph(4)-Ia-qacEdelta1-*$bla_{CTX\text{-}M65}$*-sul1*). Resistance to disinfecting agents is significant as disinfectants are used throughout the broiler production

Table 1 Antimicrobial class and genes detected in the batch sampling study

Study	Pattern	Antimicrobial class and genes detected:					
		Aminoglycoside	Antiseptics[a]	Cephalosporin	Sulphonamide	Number of isolates (%)[b]	Serovar (n, %)
Farm	A	*aac(3)-IV* *aph(3')-Ia* *aph(4)-Ia*	*qacEdelta1*	$bla_{CTX\text{-}M\text{-}65}$	*sul1*	5	Infantis(5, 100)
	B	*aac(3)-IV* *aph(4)-Ia*	*qacEdelta1*	$bla_{CTX\text{-}M\text{-}65}$	*sul1*	1	Infantis (1, 100)
Processing plant	A	*aac(3)-IV* *aph(3')-Ia* *aph(4)-Ia*	*qacEdelta1*	$bla_{CTX\text{-}M\text{-}65}$	*sul1*	1	Infantis (1, 100)
	B	*aac(3)-IV* *aph(4)-Ia*	*qacEdelta1*	$bla_{CTX\text{-}M\text{-}65}$	*sul1*	1	Infantis (1, 100)
Retail outlet	A	*aac(3)-IV* *aph(3')-Ia* *aph(4)-Ia*	*qacEdelta1*	$bla_{CTX\text{-}M\text{-}65}$	*sul1*	3	Infantis (3, 100)
	C[c]	*aac(6')-Iaa*	–	–	*sul2*	1	Typhimurium (1, 100)

[a]*qacEdelta1* is a resistance gene conferring resistance to quaternary ammonium compound (QAC) antiseptics, conferring resistance to the following drug classes: disinfecting agents, intercalating dyes, and acridine dye

[b]All isolates except *S.* Kentucky contained resistance genes (n = 1)

[c]Resistance genes *mdsA*, *mdsB*, and *sdiA* were also detected

chain in the country and elsewhere to limit cross-contamination. In our study, 81.8% (9/11) of our Infantis strains exhibited the genotypic resistant pattern *aac(3)-IV-aph(3')la-aph(4)la-bla*$_{CTX-M65}$*-sul1* (Pattern A) and 18.2% (2/11) exhibited *aac(3)-IV- aph(4)la-bla*$_{CTX-M65}$*-sul1* (Pattern B). Both patterns (A and B) were also detected in an earlier cross-sectional study, exhibiting 36.4% (4/11) and 54.5% (6/11) multi drug resistance (MDR) among *S.* Infantis strains, respectively (Khan et al. 2022b).

A significant finding of the phenotypic resistance testing was detecting a 91.8% (41/48) overall prevalence of resistance to the extended-spectrum cephalosporin and ceftriaxone. This phenotypic finding correlated with the genotypic detection of *bla*$_{CTX-M65}$ in all tested isolates of serovar Infantis. Additionally, all Infantis strains in the current study genotypically exhibited multi-drug resistance to aminoglycosides, cephalosporins, sulfonamides, and disinfecting agents (quaternary ammonium compounds and dyes). Of these 11 MDR Infantis strains, 54.5% (6/11), 18.2% (2/11), and 27.3% (3/11) were detected at the farm, processing plant, and retail outlet, respectively. In a previously published cross-sectional molecular study, among 20 detected Infantis strains, 50% (10/20) exhibited MDR (using the same panel of antimicrobial agents) containing the *bla*$_{CTX-M-65}$ gene detected at broiler farms (Khan et al. 2022b).

Regarding *S*. Infantis phylogeny, 2 distinct clusters were observed (Fig. 1). Well-supported clustering was observed within the farm, processing plant, and retail outlet for serovar Infantis isolates. Within this cluster, SAMN16678586 and SAMN16678584 were on the same branch (Fig. 1). A significant finding was the reliable clustering in all isolates from the 3 sampling stages to SAMN18407134, isolated at a USA processing plant in 2021, and 3 Infantis isolates detected at retail outlets in Barbados in 2019 (SAMN15964920, SAMN15964921, and SAMN15964932) (Supplementary Material, Table S3). The human clinical isolate was clustered with a Brazilian processing plant isolate detected in 2016 and a retail outlet sample isolated in China in 2014, but no clustering was observed to any of our isolates. This human strain was the only sequence available to have originated from Trinidad and Tobago and was included to compare *Salmonella* strains detected along the broiler production chain to human salmonellosis. In this study, no clustering was observed among the isolates and the reference strain (Infantis_strain_119944).

The MDR Infantis strains detected in this batch sampling study and cross-sectional studies (Khan et al. 2022b) contained a large plasmid ESI (pESI) as evidenced by the NCBI Pathogen detection browser,[Footnote 1] where 8 strains from this study were highly related to the MDR emergent *S.* Infantis strains carrying the *bla*$_{CTX-M-65}$ gene.[Footnote 2] This large plasmid contains several antimicrobial resistances, metal, and virulence genes found in the emergent *S.* Infantis strain reported in several countries, including South America (Cartelle Gestal et al. 2016; Palma et al. 2017) and the United States (Tyson et al. 2021). However, the plasmid detected in our *S.* Infantis carrying the CTX-M-65 gene (Accession: CP066336.1) contained 312,952 bp, differing from the plasmids reported in the USA which ranged from 316,160 to 323,122 bp (Maguire et al. 2022). Additionally, all Infantis strains in this study contained virulence factors *csg, bcf, lpf,* TTSS (SPI-1 encode), TTSS (SPI-2 encode) and TTSS-1 translocated effectors.

This study highlighted the antimicrobial resistance patterns, and phylogenies exhibited by serovar Infantis detected at different sampling levels. The findings of this study proved the transmission of serovar Infantis from farm through the processing plant to the retail outlets, posing a threat to consumers of contaminated broiler meat in Trinidad and Tobago. However, to further demonstrate the validity and application of this approach, it is imperative to conduct a more extensive study, using a higher number of farms and samples.

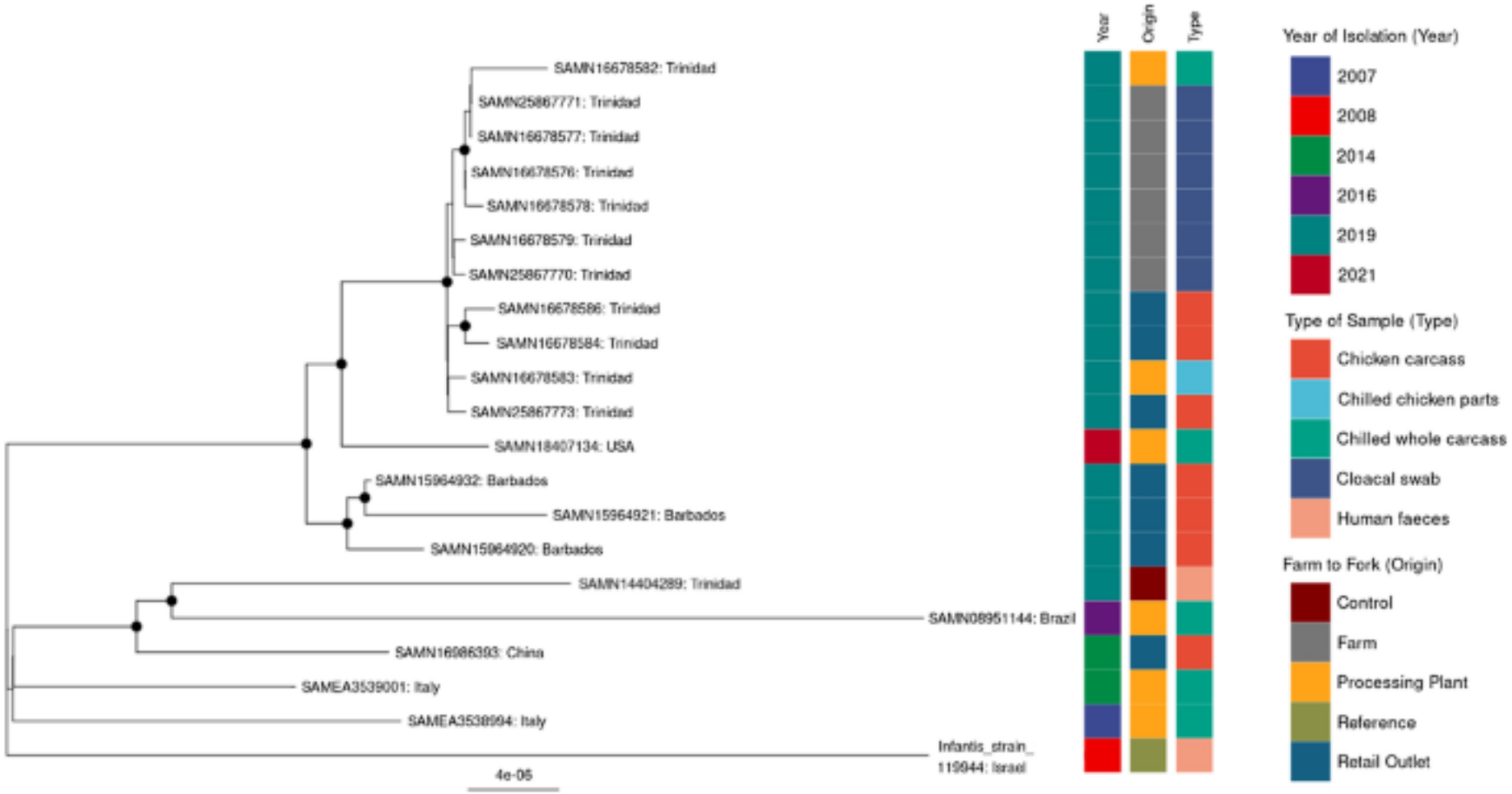

Fig. 1. Phylogenetic analysis of *S*. Infantis. Shown is a maximum-likelihood phylogeny of *S*. Infantis calculated using a core genome alignment with IQ-TREE v.2.1.2 and 1000 bootstraps specified. A black dot on the node indicates bootstrap values > 70%

Notes

1. https://www.ncbi.nlm.nih.gov/pathogens. Accessed March 18, 2022
2. https://www.ncbi.nlm.nih.gov/pathogens/tree/#Salmonella/PDG000000002.2411/PDS000089910.165?accessions=PDT000880734.1. Accessed March 18, 2022

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Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Institutional review board statement

The study was approved and conducted under terms approved by the University of the West Indies, St. Augustine Campus Research Committee (Research grant #2660–457522 on 13 April 2016). The UWI St. Augustine Campus Ethics Committee exempted the project from ethical review after assessing the research proposal.

Informed consent statement

Written informed consent has been obtained from the patient(s) by the Caribbean Public Health Agency (CARPHA) from where isolates of *Salmonella* Infantis originated to publish this paper.

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