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GENOME SEQUENCES

Whole-Genome Sequencing Analysis of *Salmonella* Isolates from Poultry Farms, a Slaughterhouse, and Retail Stalls in Thailand

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ABSTRACT The draft genome sequences of 21 *Salmonella* isolates obtained from poultry production chains in Hat Yai City, Songkhla Province, Thailand, are reported in this study. Our study revealed that there was *Salmonella* environmental contamination along poultry production chains and cross-contamination among poultry through inanimate surfaces in the environment.

In Thailand, *Salmonella* is one of the common causes of foodborne infections (1), and poultry is the most consumed meat (2). Studies have shown that *Salmonella* contamination of poultry meat can occur during processing along the production chain (3). To understand the possible sources of *Salmonella* contamination, our study aimed to characterize *Salmonella* isolates from poultry production chains in Thailand using whole-genome sequencing analysis.

In 2016, 163 samples from Hat Yai City, Songkhla Province, Thailand, were collected—environmental samples from broiler farms ($n = 32$), environmental samples from a broiler slaughterhouse ($n = 47$), and environmental and raw chicken meat samples from wet market retail stalls ($n = 84$). A total of 56 *Salmonella* isolates were recovered from the samples using the ISO 6579:2007 protocol with slight modifications (4). Randomly selected *Salmonella* isolates (one colony per sample) were stored in brain heart infusion broth with 15% glycerol until further usage. Then, 21 isolates (from farms [$n = 5$], a slaughterhouse [$n = 5$], and retail stalls [$n = 11$]) were selected based on phenotypic resistance profiles and cultured in universal preenrichment broth at 37°C for 24 h, followed by DNA extraction using the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA). Library preparation was performed using the NEBNext Ultra DNA kit (New England Biolabs [NEB], USA). Samples were sequenced on a HiSeq 4000 sequencer (Illumina, San Diego, CA, USA) with 2 × 151-bp reads. All "pass filter" reads (as called using bcl2fastq version 2.20.0.422) were used in subsequent analyses. Multilocus sequence type (MLST) and resistance gene predictions were made using SRST2 version 0.2.0 (5) with MLST databases (6) from PubMLST (https://pubmlst.org) and the ARGannot resistance gene database (7). *De novo* assemblies were performed using Velvet version 1.2.10 with the VelvetOptimizer helper script version 2.2.4 (8) and a minimum contig cutoff of 500 bp, scaffolded with OPERA-LG version 2.0.6 (9), and finished with FinIS version 0.3 (10). The assembled genomes were annotated using Prokka version 1.13.3 (11) and analyzed with the following tools: *in silico* serotyping using SeqSero version 1.0 (12), identification of *Salmonella* pathogenicity islands (SPI) using

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TABLE 1 Whole-genome sequencing characterization of 21 *Salmonella* isolates recovered from samples in farms, a slaughterhouse, and retail stalls in Hat Yai, Songkhla Province, Thailand

Sample ID[a]	Source	Sample description	Predicted serotype[b]	MLST[c]	Resistance gene(s)[d]	No. of resistance genes	Plasmid replicon(s)[e]	*Salmonella* pathogenicity islands[f]
001SL	Farm	Cooling pad water	Weltevreden	365		0		SPI-2, SPI-3, SPI-4, SPI-8, C63PI
010SL	Farm	Cooling pad water	Weltevreden	365	*aac3-Ila*, *aadA*, *inuF*, bla_{TEM-1D}	4		SPI-2, SPI-3, SPI-4, SPI-8, C63PI
020SL	Farm	Fecal	Kentucky	314		0		SPI-2, SPI-3, SPI-4, SPI-8, C63PI
030SL	Farm	Feed	Potential monophasic variant of Typhimurium	34	*strA*, *strB*, *sulII*, bla_{TEM-1D}, *tetB*	5	ColpVC	SPI-2, SPI-3, C63PI
037SL	Farm	Feed	Kentucky	314		0		SPI-2, SPI-3, SPI-4, SPI-8, C63PI
038SL	Retail stall	Cutting board swab	Corvallis or Chailey	1541	*strA*, *strB*, *sulII*, *tetA*	4		SPI-1, SPI-2, SPI-3, SPI-4, SPI-8, C63PI
044SL	Retail stall	Cutting board swab	Corvallis or Chailey	1541	*qnr-S*, *strA*, *strB*, *sulII*, *tetA*	5		SPI-2, SPI-3, SPI-4, SPI-8, C63PI
052SL	Retail stall	Work table swab	Corvallis or Chailey	1541	*qnr-S*	1		SPI-1, SPI-2, SPI-3, SPI-4, SPI-8, C63PI
058SL	Retail stall	Work table swab	Kentucky	198	*aacAad*, *sulI*, bla_{TEM-1D}	3	ColpVC, IncQ1	SPI-1, SPI-2, SPI-3, SPI-4, C63PI
067SL	Retail stall	Work table swab	Altona	1549		0	IncFII(S)	SPI-2, SPI-3, SPI-4, SPI-5, SPI-13, SPI-14, C63PI
081SL	Retail stall	Cutting board swab	Corvallis or Chailey	1541	*qnr-S*, *strA*, *strB*, *sulII*	4	IncFII(S), Inc1-I(Gamma)	SPI-2, SPI-3, SPI-4, SPI-5, SPI-13, SPI-14, C63PI
082SL	Retail stall	Cutting board swab	Corvallis or Chailey	1541	*qnr-S*, *strA*, *strB*, *sulII*	4		SPI-1, SPI-3, SPI-4, C63PI
089SL	Retail stall	Carcass washing water	Corvallis or Chailey	1541	*qnr-S*, *strA*, *strB*, *sulII*	4		SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-13, SPI-14, C63PI
095SL	Retail stall	Knife swab	Corvallis or Chailey	1541	*qnr-S*	1		
099SL	Retail stall	Work table swab	Kentucky	198	*aacAad*, *sulI*, bla_{TEM-1D}	3		SPI-1, SPI-3, SPI-4, C63PI
125SL	Retail stall	Chicken meat	Agona	13		0		SPI-1, SPI-3, SPI-8, C63PI
150SL	Slaughterhouse	Bucket swab	Kentucky	198	*aacAad*, *sulI*, bla_{TEM-1D}, *tetA*	4		SPI-2, SPI-3, SPI-4, C63PI
154SL	Slaughterhouse	Feather separation swab	Singapore	462	bla_{CMY}, *erm42*, *strA*, *strB*, *sulII*, *tetA*	6		SPI-1, SPI-3, SPI-4, SPI-5, SPI-13, SPI-14, C63PI
159SL	Slaughterhouse	Chicken pen swab	Kentucky	198	*aacAad*, *sulI*, bla_{TEM-1D}, *tetA*	4		SPI-1, SPI-2, SPI-3, SPI-4, C63PI
170SL	Slaughterhouse	Conveyor swab	Singapore	462	bla_{CMY}, *erm42*, *strA*, *strB*, *sulII*, *tetA*	6	IncC	SPI-1, SPI-3, SPI-4, SPI-5, SPI-13, SPI-14, C63PI
179SL	Slaughterhouse	Wastewater	Corvallis or Chailey	1541	*qnr-S*	1	IncQ1	SPI-1, SPI-2, SPI-3, SPI-4, SPI-8, C63PI

[a] A prefix of "SGEHI2016-PSU-BS-" applies for all isolate IDs.
[b] Using SeqSero version 1.0.
[c] Using MLST databases from https://pubmlst.org/.
[d] Using the ARGannot resistance gene database supplied with SRST2.
[e] Using PlasmidFinder version 2.1 with a minimum identity of 95% and minimum coverage of 60%.
[f] Using SPIFinder version 1.0 with a threshold ID of 95% and minimum length of 60%.

SPIFinder version 1.0, and identification of plasmid replicons using PlasmidFinder version 2.1 (Centre for Genomic Epidemiology, Denmark) (13). Upon submission to GenBank, assemblies were reannotated using the NCBI Prokaryotic Genome Annotation Pipeline (14). Default parameters for software were used except where otherwise noted.

The draft genome sizes ranged from 4,661,649 to 5,100,460 bp with GC contents of 51.90 to 52.27% (Table 1). The number of contigs for each isolate ranged from 25 to 80. Sequence type 1541 (ST1541) (*Salmonella enterica* serovar Corvallis or *Salmonella enterica* serovar Chailey) was the most commonly predicted serotype (38.1%, 8/21). Out of 21 isolates, 16 (76.2%) were predicted to carry at least one resistance gene. Five types of plasmid replicons were found in 28.6% (6/21) of isolates. All isolates (except isolate SGEHI2016-PSU-BS-095SL) contained at least 3 types of SPIs. Our study suggested that *Salmonella* contamination had occurred in the environment along poultry production chains and that there was cross-contamination among poultry through environmental surfaces. Furthermore, our study provided baseline information on the genomic diversity of *Salmonella* isolates found in the poultry production chains in Thailand.

Data availability. The raw reads and assembled genomes were deposited in GenBank under BioProject number PRJNA644105. The accession numbers for the individual isolates are listed in Table 1.

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TABLE 1 (Continued)

Total no. of sequence reads	N_{50} length (bp)	No. of contigs	GC content (%)	Total length (bp)	Total sequence data (bp)	Genomic coverage (×)	SRA no.	GenBank accession no.
3,416,142	126,091	76	52.10	5,100,460	1,031,674,884	202	SRR12151691	JADDII000000000.1
3,534,574	149,448	80	52.09	5,102,068	1,067,441,348	209	SRR12151690	JADDIH000000000.1
4,035,271	729,478	28	52.16	4,661,649	1,218,651,842	261	SRR12151679	JADDIG000000000.1
3,641,947	278,606	53	52.15	4,958,996	1,099,867,994	222	SRR12151677	JADDIF000000000.1
3,669,548	729,466	28	52.16	4,662,016	1,108,203,496	238	SRR12151676	JADDIE000000000.1
3,951,543	793,388	30	51.91	4,881,101	1,193,365,986	244	SRR12151675	JADDID000000000.1
4,137,978	793,383	32	51.91	4,890,864	1,249,669,356	256	SRR12151674	JADDIC000000000.1
3,779,794	793,530	29	51.90	4,881,258	1,141,497,788	234	SRR12151673	JADDIB000000000.1
3,454,419	817,359	31	52.21	4,880,320	1,043,234,538	214	SRR12151672	JADDIA000000000.1
3,903,860	547,503	25	52.27	4,688,587	1,178,965,720	251	SRR12151671	JADDHZ000000000.1
3,791,130	412,818	33	51.91	4,890,404	1,144,921,260	234	SRR12151689	JADDHY000000000.1
4,403,614	793,373	32	51.91	4,891,065	1,329,891,428	272	SRR12151688	JADDHX000000000.1
3,779,688	521,418	36	51.91	4,892,070	1,141,465,776	233	SRR12151687	JADDHW000000000.1
3,435,933	521,294	40	51.91	4,877,861	1,037,651,766	213	SRR12151686	JADDHV000000000.1
3,311,371	818,086	33	52.22	4,891,040	1,000,034,042	204	SRR12151685	JADDHU000000000.1
3,683,522	307,945	38	51.95	4,833,485	1,112,423,644	230	SRR12151684	JADDHT000000000.1
3,814,780	773,805	33	52.20	4,800,040	1,152,063,560	240	SRR12151683	JADDHS000000000.1
3,478,832	617,727	32	52.20	4,824,749	1,050,607,264	218	SRR12151682	JADDHR000000000.1
3,844,880	773,582	30	52.20	4,799,895	1,161,153,760	242	SRR12151681	JADDHQ000000000.1
4,116,456	466,641	33	52.20	4,824,348	1,243,169,712	258	SRR12151680	JADDHP000000000.1
3,944,301	743,296	28	51.96	4,845,458	1,191,178,902	246	SRR12151678	JADDHO000000000.1

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K.V., K.T.A., and L.C.N. conceived the study. K.V. collected the environmental and raw meat samples and performed bacterial isolation and identification from the samples. S.L.C., K.H.O., and K.T.A. performed the genomic and data analyses. K.H.O. and S.C.M.C. drafted the manuscript, and all authors were involved in the review and editing of the manuscript.

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