

Genomic insight of *Salmonella enterica* serovar Enteritidis strain HC-2sh isolated from a private poultry slaughterhouse in Korea[§]

Seunghui Lee^{1†} , Seon Young Park^{2†} , Tae Seon Cha¹ , Soojin Lim¹ , Eun Jeong Park¹ , Jong Beom Na¹ ,
Keeman Lee¹ , Ye Bin Kim¹ , Seojeong Choi¹ , Yejin Seo¹ , Jee Eun Han^{3*} , and Ji Hyung Kim^{1*} 

¹Department of Food Science and Bioengineering, College of Bionano Technology, Gachon University, Seongnam 13120, Republic of Korea

²Veterinary Drugs and Biologics Division, Animal and Plant Quarantine Agency, Gimcheon 39660, Republic of Korea

³Institute for Veterinary Biomedical Science, Department of Veterinary Medicine, Kyungpook National University, Daegu 41566, Republic of Korea

(Received July 28, 2025; Revised September 1, 2025; Accepted September 2, 2025)

Salmonella enterica serotype Enteritidis (SE) is the leading cause of foodborne outbreaks in Korea, yet genomic profiles of pathogenic Sequence Type (ST) 11 SE strains from poultry slaughterhouses remain limited. We report the ST11 SE strain's HC-2sh complete genome from a Korean poultry slaughterhouse, showing antimicrobial resistance genes (*aac* (6')-*Iaa-bla*_{TEM1b}-*dfrA1*), matching previous SE isolates from Korean retail chicken meat, and a unique virulence gene profile (*sef-pef-fim-sop-inv-org-sip-spa-sif-hil-spr-ssa-sse-prg-pil-spv*), which differs from the patterns previously reported for various SE isolates in Korea. These results provide insights into ST11 SE, which is prevalent in Korean poultry facilities, and enhance understanding of salmonellosis in the food industry.

Keywords: SE, ST11, poultry slaughterhouse, unique virulence gene profile

Invasive *Salmonella enterica* infection is a serious condition, characterized by high morbidity and mortality rates worldwide (Kang et al., 2024b). *Salmonella enterica* strains are facultative intracellular pathogens causing localized and systemic infections in humans and other vertebrates owing to genes in *Salmonella* pathogenicity islands (SPIs), prophage,

or virulence plasmids (Guiney and Fierer, 2011). To date, *S. enterica* serotype Enteritidis (SE) is commonly isolated from human patients with salmonellosis resulting from the consumption of contaminated poultry products and eggs, showing increased antimicrobial resistance (AMR) (La et al., 2021). In Korea, SE is a dominant serotype in *Salmonella* outbreaks, with Sequence Type 11 (ST11) predominating in poultry and humans (Kim et al., 2012). Poultry slaughterhouses are critical contamination points through equipment, transport crates, water, and direct contact (Kim et al., 2025). However, comprehensive genomic data from SE poultry slaughterhouse isolates are limited. We report the complete genome of an SE strain isolated from a poultry slaughterhouse with unique virulence- and AMR-associated gene profiles distinct from Korean poultry and human isolates.

Strain HC-2sh was isolated from a sterile swab at a private poultry slaughterhouse in Jeonbuk-do, Korea. The isolate was cultured overnight on tryptic soy agar (Difco) at 37°C and identified as *S. enterica* subsp. *enterica* through *gyrB*-based phylogenetic analysis (Fukushima et al., 2002) (Fig. 1A). Then, the DNA of the strain HC-2sh was extracted using the DNeasy Blood & Tissue Kit (Qiagen) and sequenced using a hybrid approach (Kwon et al., 2023). The PacBio RSII System (Pacific Biosciences) was used to construct a 20-kb SMRTbell™ template library. The HiSeq X-10 platform (Illumina) was used to prepare a DNA library using a TruSeq Nano DNA Library Prep Kit (Illumina). The Microbial

[†]These authors contributed equally to this work.

*For correspondence. (J.H. Kim) E-mail: kzh81@gachon.ac.kr;

Tel.: +82-31-750-5383; Fax: +82-31-750-5389 /

(J.E. Han) E-mail: jehan@knu.ac.kr;

Tel.: +82-53-950-5972; Fax: +82-53-950-5955

[§]Supplemental material for this article may be found at
<http://www.kjom.org/main.html>

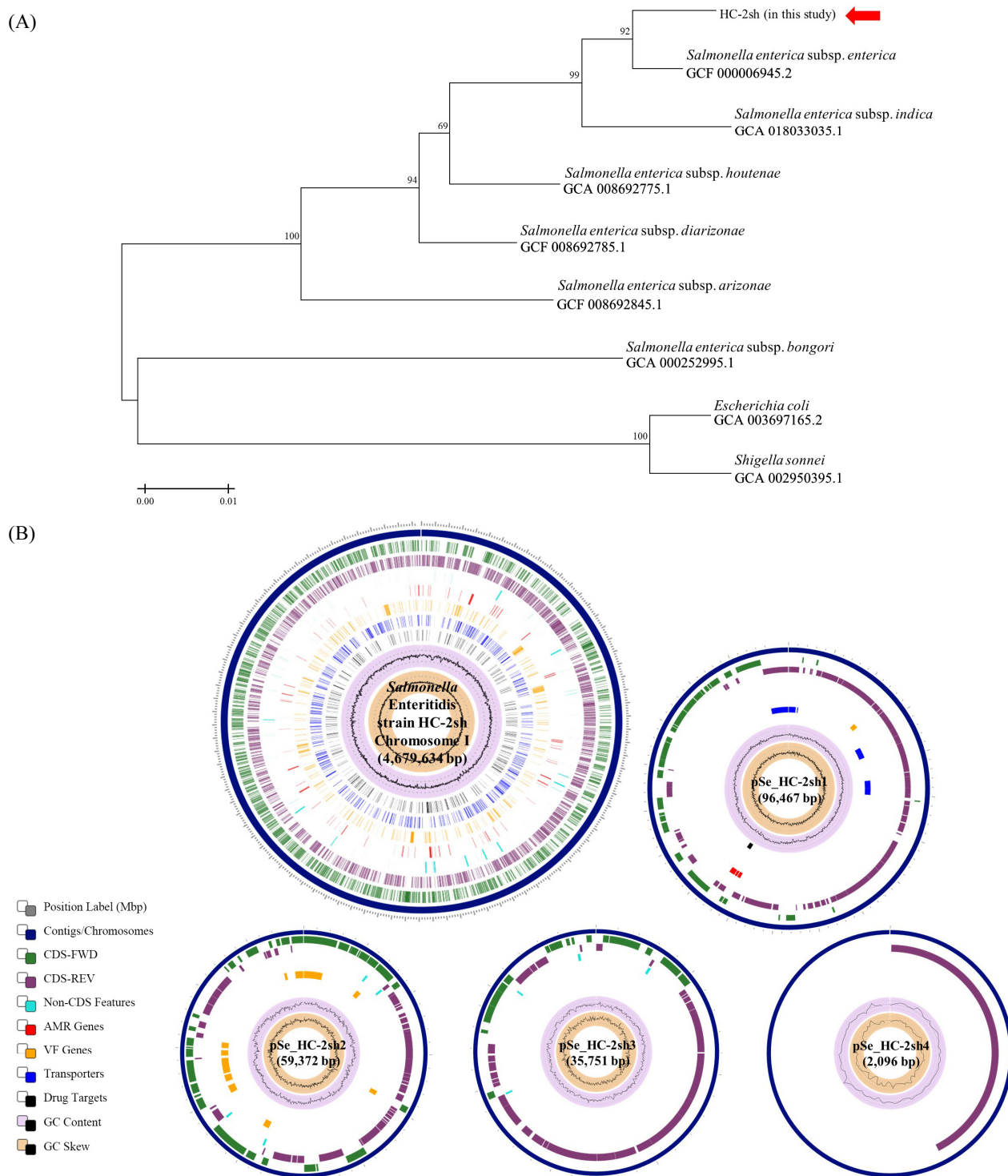


Fig. 1. The *gyrB* gene-based phylogeny of SE strain HC-2sh (A). The Neighbor-Joining phylogenetic tree was reconstructed based on the *gyrB* gene sequences of representative reference genomes of Enterobacteriaceae available in the GenBank database using MEGA 11 (Tamura et al., 2021) with 1,000 bootstrap replicates. The strain HC-2sh was well clustered with *S. enterica* subsp. *enterica* (GCF_000006945.2) showing 0.002 genetic distance and 98.4% sequence similarity with the strain. Bar: 0.02 substitutions per nucleotide position. The genome map of SE strain HC-2sh (B). The genome was visualized and classified into subsystems using the Comprehensive Genome Analysis of Pathosystems Resource Integration Center (PATRIC) server v.3.6.12 (Olson et al., 2023). CDS-FWD, coding sequences on the forward strand; CDS-REV, coding sequences on the reverse strand; AMR, antimicrobial-resistance genes; VF, virulence factors.

Table 1. General genomic features of the ST11 SE strain HC-2sh

Features	Value
Genome size (bp)	4,873,320
Chromosome I	4,679,634
Plasmid (pSe_HC-2sh1)	96,467
Plasmid (pSe_HC-2sh2)	59,372
Plasmid (pSe_HC-2sh3)	35,751
Plasmid (pSe_HC-2sh4)	2,096
G + C content (%)	52.08
Coverage (total)	235.94 ×
No. of contigs	5
Chromosomes	1
Plasmids	4
Total genes	4,806
Protein-coding genes	4,494
tRNAs	83
rRNAs	22
ncRNAs	11
Pseudogenes	192

Genome Assembly application (<http://www.pacb.com/products-and-services/analytical-software/smrt-analysis/>), incorporating the Hierarchical Genome Assembly Process (HGAP v3.0), was used for hybrid assembly of PacBio long reads (1,647,883,573 bp; 363,519 reads) and Illumina paired-end reads (1,194,155,283 bp; 7,915,890 reads). The assembled genome was polished for error correction using Pilon v1.21 (Walker et al., 2014). Genome annotation was performed using the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

The sequenced HC-2sh genome comprised 4,873,320 bp (52.1% G + C content, 235.9 × coverage) with five circular contigs, one chromosome, and four plasmids encoding 4,686 coding DNA sequences (CDSs), 83 tRNAs, and 22 rRNAs. The chromosome was 4,679,634 bp long [52.0% G + C, 4,449 coding sequences (CDSs)]. Plasmid pSe_HC-2sh1 was 96,467 bp long (51.5% G + C, and 108 CDSs). The pSe_HC-2sh2 plasmid was 59,372 bp long (52.0% G + C, 80 CDSs), the pSe_HC-2sh3 plasmid was 35,751 bp long (41.0% G + C, 48 CDSs), and the pSe_HC-2sh4 plasmid was 2,096 bp long (55.5% G + C, 1 CDS) (Table 1). Furthermore, the graphical genome maps were aligned and visualized using the Bacterial

and Viral Bioinformatics Resource Center (BV-BRC) server 3.46.3 (Olson et al., 2023) (Fig. 1B).

Genomic similarities between strain HC-2sh and seven *S. enterica* reference strains were assessed using the Orthologous ANI tool software (Lee et al., 2016). The analysis revealed that the genome of strain HC-2sh exhibited the highest average nucleotide identity (ANI) value (> 99.9%) compared to the reference SE strain P125109 (accession number AM933172) (Fig. S1A). The serotype and O: H antigen type of strain HC-2sh were determined in silico using SeqSero software from the Center for Genomic Epidemiology (Zhang et al., 2019), and the results indicated that the isolate was serovar Enteritidis with a 9: g, m: - antigenic formula (O antigen: 9, H1 antigen: g, m, H2 antigen: -). Multilocus sequence typing (MLST) was performed to determine the ST of strain HC-2sh. The sequences of seven housekeeping genes (*aroC*, *dhnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*) from the sequenced genome were compared in silico with those available in the PubMLST database (<https://pubmlst.org/organisms/salmonella-spp>). The allele profile of the strain was identified as 5-2-3-7-6-6-11, corresponding to ST11. Replicon sequence typing of the plasmid was performed using PlasmidFinder 2.1 and pMLST 2.0 (Carattoli et al., 2014). The pSe_HC-2sh1 plasmid was classified as Incompatibility group I1 (IncI1-α). Plasmid pSe_HC-2sh2 harbored multiple replicon types, including IncFIB and IncFIIS. Finally, plasmids pSe_HC-2sh3 and pSe_HC-2sh4 were classified as IncX1 and ColpVC replicons, respectively. The four plasmids in the HC-2sh genome were further compared with its relative plasmids, which were identified by BLASTn searches against the NCBI nt database with default parameters, and the plasmids pSe_HC-2sh1 and pSe_HC-2sh2 were visualized using the BLAST Ring Image Generator (BRIG) (Fig. S1B and S1C).

Plasmids pSe_HC-2sh1 and pSe_HC-2sh3 were respectively matched to plasmids pZ01320SL0031_1 (CP087507.1) and pZ01319SL0003_3 (CP087557.1), which were previously isolated from Korean chicken meat, suggesting transmission between SE strains in poultry products and slaughterhouses. Notably, plasmid pSe_HC-2sh3 encoded conjugative secretion- and conjugation-related genes with low G + C contents (< 40%), which are often encoded on a self-transmissible

plasmid (Wallden et al., 2010), thus contributing to its overall low G + C contents (41.0%). The pSe_HC-2sh2 plasmid showed high genomic similarity (> 99%) with several IncFIB/IncFIIS-type virulence plasmids of different geographical origins, suggesting its potential to confer pathogenicity to humans (Fig. S1C). The plasmid pSe_HC-2sh4 showed the highest genomic similarity (99.9%) with the plasmid pN18S 0035-3 (CP082613.1) of turkey product-isolated SE in the USA; however, virulence- and AMR-associated genes were not detected in the genomes of plasmids pSe_HC-2sh3 and pSe_HC-2sh4.

Potential AMR- and virulence-associated genes in the strain HC-2sh were screened as previously described (Kwon et al., 2023). Three AMR-associated genes were detected in strain HC-2sh: *aac(6')-Iaa* (chromosome) and *bla_{TEM1b}* and *df_rA1* (pSe_HC-2sh1 plasmid), conferring resistance to aminoglycosides, beta-lactams, and trimethoprim, respectively. The pSe_HC-2sh1 plasmid uniquely harbored *bla_{TEM1b}* and *df_rA* genes, absent in the available IncI1-(α) type reference plasmids in GenBank (Fig. S1B, Table S1). In total, 135 virulence genes were identified in HC-2sh, including 80 identical SPI-associated genes. The virulence plasmid pSe_HC-2sh2 contained virulence factors related to Fimbrial adherence determinants (*pef*), Macrophage-inducibility (*mig-5*), Serum resistance (*reck*), and a conserved *Salmonella* plasmid virulence (*spv*) operon (~8 kb) consisting of *spvR* and *spvA-D*. In *Salmonella* pathogenesis, *spv* is involved in replication in extra-intestinal sites, such as the liver and spleen, and is associated with severe and disseminated non-typhoid *Salmonella* infections in humans (Guiney and Fierer, 2011; Kang et al., 2024b). The *tra* (transfer gene) operon (*traM-traJ-traY-traA-traL*), which mediates the physical transfer of plasmids between bacterial cells (Oluwadare et al., 2020), was also detected (Fig. S1C, Table S2). HC-2sh exhibited a unique virulence-associated gene profile (*sef-pef-fim-sop-inv-org-sip-spa-sif-hil-spr-ssa-sse-prg-pil-spv*), which differed from the patterns previously reported for various Korean SE isolates (Hyeon et al., 2021; Kang et al., 2024a; La et al., 2021) by the addition of *sef*, *spr*, *pil*, and *spv* genes. The AMR-associated gene pattern of strain HC-2sh (*aac(6')-Iaa-bla_{TEM1b}-df_rA1*) was similar to that previously described for SE isolates from retail chicken meat in Korea

(La et al., 2021). In addition, four prophage regions were detected in the genome using the PHASTEST server (Wishart et al., 2023), including two intact prophage regions (score > 90) and two incomplete regions (score < 70). The intact prophage regions (Region 1; 64.3 kb a, Region 3; 31.1 kb) were similar to those of *Salmonella* phage 118970_sal3 (NC_031940.1) and *Salmonella* phage Gifsy-2 (NC_010393.1), respectively. Notably, the type III secretion system effector protease (GtgA; 3965387-3965860) and superoxide dismutase (SodC1; 3968506-3969039), which can potentially influence the virulence of *Salmonella* (Ammendola et al., 2005; Ruano-Gallego et al., 2021), were identified within the intact prophage region 3 (Table S3).

Until recently, ST11 was one of the most frequently reported foodborne pathogens in Korea. The AMR SE strain HC-2sh isolated from a poultry slaughterhouse exhibited a unique pattern of virulence factors not previously reported in domestic SE strains, indicating the possible presence of new genomic traits that may affect pathogenicity. These results emphasize the need for genomic surveillance of slaughterhouse SE strains and food safety in poultry production chains, providing insights for control strategies against SE, which causes economic losses in Korean agriculture and public health.

Nucleotide sequence accession number

Salmonella enterica serovar Enteritidis strain HC-2sh was deposited in the Korean Culture Center of Microorganisms (KCCM) under the deposition number KCCM 90533. The complete genome sequence of strain HC-2sh has been deposited in GenBank under accession numbers CP185380 (chromosome), CP185381 (pSe_HC-2sh1), CP185382 (pSe_HC-2sh2), CP185383 (pSe_HC-2sh3), and CP185384 (pSe_HC-2sh4). The associated Bioproject, Biosample, and SRA accession numbers are PRJNA1305176, SAMN50589019, and SRR35008309, respectively.

Acknowledgments

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (RS-2024-00336046) and the Development

of Technology for the Biomaterialization of Marine Fishery Byproducts of the Korea Institute of Marine Science and Technology Promotion (KIMST), funded by the Ministry of Oceans and Fisheries (KIMST-20220128).

Conflict of Interest

All authors declare no conflicts of interest relevant to this research.

References

- Ammendola S, Ajello M, Pasquali P, Kroll JS, Langford PR, et al. 2005. Differential contribution of *sodC1* and *sodC2* to intracellular survival and pathogenicity of *Salmonella enterica* serovar Choleraesuis. *Microbes Infect.* 7: 698–707.
- Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, et al. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother.* 58: 3895–3903.
- Fukushima M, Kakinuma K, Kawaguchi R. 2002. Phylogenetic analysis of *Salmonella*, *Shigella*, and *Escherichia coli* strains on the basis of the *gyrB* gene sequence. *J Clin Microbiol.* 40: 2779–2785.
- Guiney DG, Fierer J. 2011. The role of the *spv* genes in *Salmonella* pathogenesis. *Front Microbiol.* 2: 129.
- Hyeon JY, Li S, Mann DA, Zhang S, Kim KJ, et al. 2021. Whole-genome sequencing analysis of *Salmonella enterica* serotype Enteritidis isolated from poultry sources in South Korea, 2010–2017. *Pathogens.* 10: 45.
- Kang H, Kim H, Lee J, Jeon JH, Kim S, et al. 2024a. Genetic characteristics of multidrug-resistant *Salmonella* isolated from poultry meat in South Korea. *Microorganisms.* 12: 1646.
- Kang L, You J, Li Y, Huang R, Wu S. 2024b. Effects and mechanisms of *Salmonella* plasmid virulence gene *spv* on host-regulated cell death. *Curr Microbiol.* 81: 86.
- Kim T, Kim Y, Kim H, Moon JS, Chon J, et al. 2025. Prevalence of *Salmonella* serotypes isolated from clinical samples in chicken farms and meat in slaughterhouses in South Korea. *Poult Sci.* 104: 105147.
- Kim MS, Lim TH, Jang JH, Lee DH, Kim BY, et al. 2012. Prevalence and antimicrobial resistance of *Salmonella* species isolated from chicken meats produced by different integrated broiler operations in Korea. *Poult Sci.* 91: 2370–2375.
- Kwon H, Park SY, Han SY, Han JE, Kim JH. 2023. Complete genome sequence of *Edwardsiella anguillarum* strain C-5-1 isolated from diseased Giant mottled eel (*Anguilla marmorata*) cultured in Korea. *Korean J Microbiol.* 59: 30–33.
- La TM, Kim T, Lee HJ, Lee JB, Park SY, et al. 2021. Whole-genome analysis of multidrug-resistant *Salmonella* Enteritidis strains isolated from poultry sources in Korea. *Pathogens.* 10: 1615.
- Lee I, Kim YO, Park SC, Chun J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol.* 66: 1100–1103.
- Olson RD, Assaf R, Brettin T, Conrad N, Cucinell C, et al. 2023. Introducing the bacterial and viral bioinformatics resource center (BV-BRC): a resource combining PATRIC, IRD and ViPR. *Nucleic Acids Res.* 51: D678–D689.
- Oluwadare M, Lee MD, Grim CJ, Lipp EK, Cheng Y, et al. 2020. The role of the *Salmonella* *spvB* IncF plasmid and its resident entry exclusion gene *traS* on plasmid exclusion. *Front Microbiol.* 11: 949.
- Ruano-Gallego D, Sanchez-Garrido J, Kozik Z, Núñez-Berrueto E, Cepeda-Molero M, et al. 2021. Type III secretion system effectors form robust and flexible intracellular virulence networks. *Science.* 371: eabc9531.
- Tamura K, Stecher G, Kumar S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol.* 38: 3022–3027.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, et al. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One.* 9: e112963.
- Wallden K, Rivera-Calzada A, Waksman G. 2010. Microreview: Type IV secretion systems: versatility and diversity in function. *Cell Microbiol.* 12: 1203–1212.
- Wishart DS, Han S, Saha S, Oler E, Peters H, et al. 2023. PHASTEST: faster than PHASTER, better than PHAST. *Nucleic Acids Res.* 51: W443–W450.
- Zhang S, den Bakker HC, Li S, Chen J, Dinsmore BA, et al. 2019. SeqSero2: rapid and improved *Salmonella* serotype determination using whole-genome sequencing data. *Appl Environ Microbiol.* 85: e01746-19.