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Insights into the Genetic Diversity of Cambodian Salmonella Serovars via Whole-Genome Analysis

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Abstract

Background

Antimicrobial resistance (AMR) among *Salmonella* enterica serovars constitutes a significant challenge for food safety and human health in Cambodia. The aim of this study was to enhance current knowledge about antimicrobial-resistant genes (ARGs) and their associated virulence determinants in *Salmonella* serovars using whole-genome sequencing (WGS).

Methods

A total of 32 *Salmonella* serovars were identified among 75 isolates isolated from vegetables, meat, and seafood/fish obtained from open markets and vegetable farms in Cambodia, using seqSero2. All WGS data were analyzed using multiple antibiotic-resistance gene prediction tools (CARD-RGI, AMRFinderPlus, and ABRicate) to cross-validate ARGs detection, and virulence factor detection tools (PlasmidFinder, VFAnalyzer, ABRicate with VFDB, and SPIFinder).

Results

The three analysis tools consistently detected several common resistance genes, including *tet* (48%), *qnr* (48%), *sul* and *dfr* (40%), *aac* and *aph* (40%), bla_{TEM} (32%), as well as *floR* and *cml* (32%). Twenty-two plasmid types were identified, with the most frequent being Col(pHAD28) (53%), followed by Col440I (17%) and IncFII(S) (14%). A diverse range of virulence genes was observed, with Type Three Secretion System genes detected across all serovars. Additionally, twelve *Salmonella* Pathogenicity Islands (SPIs) were identified, five of which (SPI-1, SPI-2, SPI-3, SPI-5, and SPI-9) were consistently present in all serovars.

Conclusions

These results highlight the substantial genetic diversity of *Salmonella* strains isolated from various food products sold at local markets in Cambodia, which potentially contribute to the spread of AMR within food chains. WGS plays a key role in epidemiological investigations and is essential for ongoing genomic surveillance of Cambodia's food supply.

Background

Salmonella enterica (S. enterica), a motile, facultatively intracellular, Gram-negative bacterium, is a well-known foodborne enteric pathogen responsible for multiple outbreaks worldwide [25, 30]. This pathogen is particularly important because it can infect a wide range of hosts, including humans and animals [3]. Among bacterial foodborne pathogens, nontyphoidal *Salmonella* (NTS) is the most frequently

encountered, causing foodborne illnesses and posing a challenge to human health [35]. Increasing numbers of reports worldwide have documented the high prevalence of human salmonellosis outbreaks originating from various sources, including humans, food products, animals, and environments [21, 41, 65]. For instance, in Asia, the predominant serovars *S.* Enteritidis and *S.* Typhimurium have often been isolated from food sources [21]. In addition, antimicrobial resistance (AMR) in *Salmonella* is causing an increasing number of severe health issues.

Multidrug-resistant (MDR) *S. enterica* serovars are spreading across the globe due to the unrestricted use of antibiotics in several regions, and this has grown into a more serious health and societal threat in recent years [36]. Numerous studies have been conducted to identify *Salmonella* resistance genes and to gain a better understanding of resistance mechanisms, in line with the One Health approach, which integrates human, animal, and environmental health [32, 70, 66]. In Cambodia, however, only a small number of studies have expanded into the genetic analysis of *Salmonella*, with the majority placing a focus on the prevalence of phenotypic and genotypic antimicrobial resistance (AMR) in *Salmonella* isolates from humans, the environment, and various food sources [18, 57, 61, 62].

The various methods used to characterize *Salmonella* strains range from conventional to advanced methods [37, 38]. Culture-based methods have remained the gold standard for detecting, isolating, and characterizing *Salmonella* from food, environmental, and human sources [46, 55]. Recently, whole genome sequencing (WGS) has proved an important tool for gaining an enhanced understanding of microbial genetic characteristics, including serovar identification, antimicrobial resistance genes (ARGs), virulence factors, plasmids, and pathogenicity islands detection [16, 27, 56]. Several analytical tools, such as CARD, ResFinder, AMRFinderPlus, and Abricate, have been shown to be effective in ARG detection [11, 19, 23, 27, 31]. In addition to the analytical tools mentioned above, PlasmidFinder, VFAnalyzer, and SPIFinder provide further insights and a deeper understanding of microbial genomics [9].

While previous studies have described phenotypic AMR prevalence, the genetic basis and comparative evaluation of ARG-prediction tools for Cambodian isolates are yet to be explored. Acquiring such knowledge would provide important data for preventing foodborne salmonellosis and the spread of MDR. Notably, a study by Huoy et al. (2025) reported inconsistencies between phenotypic and genotypic AMR results when relying on a single tool for ARG detection. Therefore, this study aimed to expand on the current knowledge of ARGs and their associated virulence factors among the *Salmonella* serovars previously identified in the Cambodian food chain through comprehensive WGS analysis. This involved assessments of serovar distribution across various food commodities, as well as comparisons between tools for predicting ARGs, detecting plasmids, identifying virulent factors, and characterizing *Salmonella* pathogenicity islands (SPIs).

Methods

Salmonella isolate selection

A total of 75 *Salmonella* isolates originating from meat, seafood/fish, and fresh leafy green vegetables in Cambodia [28] were investigated in the present study. *Salmonella* isolates from these food samples were confirmed through a PCR-based molecular method, and AMR profiling and whole-genomic DNA extraction were also performed. The procedures for *Salmonella* isolate preparation, genomic DNA extraction, and WGS have been described in detail by Huoy et al. (2024) and Huoy et al. (2025).

Salmonella whole genome sequence analysis

Sequence quality control and trimming

FastQC [2] was used to assess the quality of the raw Illumina sequencing reads. Trimmomatic improved the read quality [5] by removing adapter sequences and filtering out low-quality reads (Phred score < 25) using default parameters.

Whole-Genome Assembly and Serovar Prediction

Genome assemblies from Illumina short reads were generated using SPAdes v3.15.5.

All 75 *Salmonella* WGSs were reanalyzed using SeqSero2 to obtain an accurate serovar prediction based on quality-controlled sequencing reads [71]. This tool employs a reference database specifically designed for *Salmonella* serotyping.

ARGs, plasmid, virulence factors, and pathogenicity island identification

To investigate the genetic context of resistance, virulence, and pathogenicity islands, we analyzed assembled contigs using PlasmidFinder v2.1, VFAnalyzer (VirulenceFinder v2.0.4), SPIFinder v1.0, ABRicate v1.0.1 with the VFDB (Virulence Factor Database), along with two other ARG prediction tools (CARD-RGI and AMRFinderPlus). PlasmidFinder was used to identify plasmid replicons [8]; VFAnalyzer screened for virulence genes using curated virulence factor references [42]; SPIFinder detected SPIs using a dedicated SPI reference database [60]; and ARGTOOLS identified acquired AMR genes and predicted resistance phenotypes. The Resistance Gene Identifier (RGI) utilized the Comprehensive Antibiotic Resistance Database (CARD) [1], while ABRicate and AMRFinderPlus employed the NCBI database [20, 42]. All tools were run with default thresholds (≥ 90% identity and ≥ 60% coverage, where applicable) and the most recent database versions that were available at the time of analysis. When relevant, gene and replicon positions were compared to assess co-localization on the same contig, which could suggest potential plasmid-associated resistance or virulence.

All sequence analyses were performed at the Department of Animal Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, and the Bioinformatics Data Analysis Core Facility at the Faculty of Medicine and Health Sciences, Linköping University, Linköping, Sweden.

Results

Distribution of Salmonella serovars

Among the 75 *Salmonella* isolates examined, WGS analysis identified 32 serovars (Fig. 1), 17 of which were detected in meat samples (n = 32), with *S.* Corvallis and *S.* Agona being the most common. In seafood/fish samples (n = 17), 15 distinct serovars were identified, with *S.* Livingstone and *S.* Braenderup being the most prevalent. In fresh vegetable samples (n = 26), 17 different serovars were identified, with *S.* Weltevreden emerging as the most dominant. Additionally, several serovars were identified across multiple food types. Specifically, *S.* Kentucky, *S.* Haifa, and *S.* Bareilly were detected in all food types, whereas *S.* Weltevreden, *S.* Typhimurium, *S.* Kedougou, and *S.* Molade/*S.* Wippra was found in both vegetable and seafood/fish samples.

Salmonella phenotypic and genotypic antimicrobial resistance

The number of ARGs associated with resistance to various antibiotic classes detected by CARD-RGI, AMRFinderPlus, and ABRicate is shown in Table 1. CARD-RGI detected a broader range of resistance genes, encompassing both specific resistance determinants and MDR genes. For instance, in addition to macrolide resistance genes (*mphA, msrC, Mrx*, and *ErmB*), the CPR gene, which confers resistance to multiple classes, including macrolides, fluoroquinolones, and penicillins, was also identified in macrolide-resistant strains. Regarding tetracycline resistance, CARD-RGI identified it in all 40 isolates, whereas ABRicate detected it in 31 isolates, and AMRFinderPlus in 29 isolates.

Table 1
Number and percentage of *Salmonella* strains with antimicrobial resistance and genes-associated resistance detection with three analysis tools, isolated from food products sold at local markets in Cambodia.

Antimicrobial Class	Antibiotic Agent	No. of Isolates (%)	No. of Isolates (%) with ARGs detected		
			CARD-RGI[(a)]	AMRFinderPlus	ABRicate
Macrolides	Azm	35 (47%)	35 (100%)	2 (6%)	4 (11%)
beta-lactam	Cxm, Amp, Atm, Aml, Ipm	39 (52%)	37 (95%)	20 (51%)	19 (49%)
Folate Pathway Antagonists	Sxt	18 (24%)	18 (100%)	13 (72%)	13 (72%)
Quinolones	Cip	10 (13%)	10 (100%)	5 (50%)	5 (50%)
Phenicols	C	21 (28%)	21 (100%)	16 (76%)	17 (81%)
Tetracycline	Ot, Do	40 (53%)	40 (100%)	29 (73%)	31 (78%)
Aminoglycosides	Gn	13 (17%)	13 (100%)	7 (54%)	8 (62%)
Multidrug-resistance strains		75 (100%)	75 (100%)	3 (4%)	6 (8%)

[(a)] Multidrug-resistance (MDR) genes together with genes conferring resistance to individual antibiotic classes.

A comparison of the resistance gene details provided by the three analytical tools revealed minor differences in the gene names reported by each tool (Table 2). The common resistance genes detected by all three tools were *bla*$_{TEM}$ (beta-lactam resistance), *tet* (tetracycline resistance), *qnr* (plasmid-mediated quinolone resistance), *sul* and *dfr* (folate pathway antagonist resistance), and *floR* and *cml* (phenicol resistance). However, for beta-lactam resistance genes, CARD-RGI reported gene names without the "bla" prefix, unlike both AMRFinderPlus and ABRicate, which included the "bla" prefix in their annotations. These common resistance genes were detected among *Salmonella* isolates, such as *tet* (48%), *qnr* (48%), *sul* and *dfr* (40%), *aac* and *aph* (40%), *bla*$_{TEM}$ (32%), and *floR* and *cml* (32%). MDR genes were also identified, including common genes such as *mar*A, *sdi*A, *rsm*A, *acr*B, *mdt*M, *gol*S, *mds*A, *mds*B, and *mds*C, which were detected by CARD-RGI. The common MDR genes detected by the other two tools included *oqx*A and *oqx*B genes, which were also identified in a few *Salmonella* strains by CARD.

Table 2
Comparison of antimicrobial resistance gene prediction by three tools (CARD-RGI, AMRFinderPlus, and ABRicate) in *Salmonella* isolates from food samples collected in Cambodia.

Antimicrobial classes	**Antibiotic agents**	**Antimicrobial Resistance Genes (ARGs)**		
		CARD-RGI	**AMRFinderPlus**	**ABRicate**
Beta-lactam	Aztreonam (Atm)	TEM-1, CTX-M-65, TEM-176, TEM-215, CTX-M-55, CMY-159, Sed-1, LptD, SHV-26, MdtQ, OXA-1, OXA-10, LAP-2 Haemophilus influenzae PBP3 conferring resistance to beta-lactam antibiotics.	blaTEM-1, blaCMY, blaSHV-26, blaTEM-215, blaCTX-M-55, blaTEM-176, blaCTX-M-65, blaOXA-1, blaSED, blaLAP-2, blaOXA-10	blaTEM-1, blaTEM-176, blaTEM-215, blaCTX-M-55, blaOKP-B-3, blaLAP-1, blaLAP-2, blaCMY-40, blaCMY-100, blaCTX-M-65, blaOKP-B-9, blaSED-1, blaSHV-26, blaACT-20, blaACT-31, blaACT-41, blaOXA-1, blaOXA-10
	Cefuroxime (Cxm)			
	Imipenem (Ipm)			
	Ampicillin (Amp)			
	Amoxicillin (Aml)			
Tetracycline	Oxytetracycline (Ot)	tet(A), tet(B), tet(M), tet(45), tet(X4), tetR, adeF, E.coli mdfA	tet(A), tet(B), tet(L), tet(M), tet(X4)	tet(A), tet(B), tet(L), tet(M), tet(J), tet(S), tet(X4), tet(34)
	Doxycycline (Do)			
Aminoglycosides	Gentamycin (Gn)	AAC(3)-IIe, AAC(3)-IVa, AAC(6')-Iaa, AAC(6')-Ib10, AAC(6')-Ii, AAC(6')-Iid, AAC(6')-Iy, APH(3')-Ia, APH(3")-Ib, APH(4)-Ia, APH(6)-Id aadA, aadA2, aadA3, aadA7, aadA16, aadA23 acrD, baeR, cpxA, kdpE	aadA1, aadA2, aadA7, aadA16, aadA22, aac(6')-I, aac(3)-Id, aac(3)-Iva, aac(3)-Iid, aac(3)-Iie, aac(6')-Ib-cr5, aph(3')-Ia, aph(3")-Ib, aph(6)-Id, aph(4)-Ia	aac(6')-I, aac(6')-If, aac(3)-Iia, aac(3)-Id, aac(6'), aac(6')-Iid, aac(3)-Iva, aph(3")-Ib, aph(6)-Id, aph(3')-Ia, aph(4)-Ia, aadA1, aadA2, aadA7, aadA8, aadA12, aadA15, aadA16, aadA22, A7J11_02581,
Quinolone/ Fluoroquinolone	Ciprofloxacin (Cip)	emrA, emrB, emrR, MdtK, QnrS1, QnrS2, QnrD1, QepA2, QnrB19,	qnrS, qnrS1, qnrS2, qnrD1, qnrB12, qnrB19, qepA1	qnrS1, qnrS2, qnrD1, qnrB11, qnrB12, qnrB19, qepA1

Antimicrobial classes	Antibiotic agents	Antimicrobial Resistance Genes (ARGs)		
		CARD-RGI	**AMRFinderPlus**	**ABRicate**
		E.coli parC, S. isangi gyrA, *Salmonella* enterica gyrA		
Phenicols	Chloramphenicol (C)	floR, catB3, cmlA1, cmlA5, catII from *E.* Coli K-12	floR, catA2, catB3, cmlA1, cmlA5,	floR, cmlA, cmlA1, cmlA5, catA, catA2, catB3
Folate Pathway Antagonists	Sulfamethoxazole-Trimethoprim (Sxt)	sul1, sul2, sul3, dfrA1, dfrA12, dfrA14	sul1, sul2, sul3, dfrA1, dfrA12, dfrA14, dfrA27,	sul1, sul2, sul3, dfrA1, dfrA12, dfrA14, dfrA27, dfrE
Macrolides	Azithromycin (Azm)	mphA, msrC, Mrx, ErmB, CRP	mph(A), erm(B), msr (C), lsa (A)	mef(B), mph(A), msr(C), eat(A), erm(B)
MDR genes		marA, sdiA, rsmA, acrB, mdtM, golS, mdsA, mdsB, mdsC, oqxA, oqxB *E.* Coli (mdfA, acrA, soxS mutation, soxR mutation, AcrAB-TolC with AcrR mutation, AcrAB-TolC with MarR mutation) KpnE, KpnF, KpnH	mdsA, mdsB, oqxA10, oqxB19, oqxA, oqxB	oqxA, oqxA3, oqxA9, oqxA10, oqxB, oqxB5, oqxB9, oqxB11, oqxB12, oqxB14, oqxB17, oqxB19, oqxB24, oqxB28

Distribution of plasmid replicons

PlasmidFinder analysis identified 22 plasmids across 32 *Salmonella* serovars, with two major plasmids belonging to Col and Inc plasmids (Table 3). The Col plasmid comprised six subtypes, namely Col(pHAD28), Col440I, Col3M, ColpVC, Col (BS512), and ColRNAI, while Inc plasmids included IncC, IncF, IncH, IncR, IncX, and IncY. Col(pHAD28) showed the greatest prevalence, detected in 23 serovars, followed by Col440I in 10 serovars and IncFII(S) in 6 serovars. The IncFII(p96A) plasmid was exclusively identified in *S.* Haifa and is associated with the horizontal transfer of resistance genes among *Enterobacteriaceae*, including *Salmonella* species. Additionally, of the 75 *Salmonella* isolates analyzed in

this study, 13 showed no detectable known plasmids. These consisted of three isolates of *S.* Livingstone, two isolates of *S.* Hvittingfoss, and one isolate each of *S.* London, *S.* Thompson, *S.* Bareilly, *S.* Newport, *S.* Potsdam, *S.* Aberdeen, *S.* Mkamba, and *S.* Wa.

Table 3
Plasmid distribution in different *Salmonella* serovars isolated from food samples in Cambodia.

Serovars	**Plasmid amplicons**
S. Corvallis	Col(pHAD28), Col440I, IncFIB(K)(pCAV1099-114), IncX1/X2
S. Haifa	Col(BS512), Col(pHAD28), Col440I, IncFII(p96A), IncFII(S), IncHI2, IncHI2A, IncY
S. Agona	Col(pHAD28), IncC, IncX1/X2
S. Kentucky	Col(pHAD28), Col3M, ColpVC, IncR, p0111, rep1
S. Livingstone	Col(pHAD28)
S. Typhimurium	Col(pHAD28), Col440I, ColRNAI, IncFIB(S), IncFII(S)
S. Uganda	Col(pHAD28), Col440I, IncQ1/Q2
S. infantis	Col(pHAD28), Col440I, IncFIB(K)(pCAV1099-114), IncFIB(pN55391), rep1
S. Rissen	Col(pHAD28), repUS42
S. Weltevreden	Col(pHAD28), ColRNAI, IncFIB(S), IncFII(S)
S. Hvittingfoss	ColpVC, IncQ1/Q2
S. Bareilly	Col(pHAD28), Col440I, IncFII(S)
S. Mbandaka	Col(pHAD28), Col440I, ColRNAI, IncFIB(K)(pCAV1099-114), IncFIB(S), IncFII(S), IncHI2, IncHI2A, IncR
S. Anatum	Col(pHAD28), IncX1/X2, IncY
S. London	Col(BS512), Col(pHAD28)
S. Kedougou	Col(IMGS31), Col(pHAD28), Col440I, ColRNAI, IncFIB(K)(pCAV1099-114)
S. Thompson	Col(pHAD28)
S. Braenderup	Col440I, IncQ1/Q2
S. Molade / *S*. Wippra	Col(pHAD28)
S. Indiana	Col(pHAD28), Col440I, IncQ1/Q2
S. Give	Col(pHAD28)
S. Heidelberg	Col3M
S. Newport	
S. Brancaster	Col(pHAD28), ColRNAI, IncX1/X2
S. Johannesburg	Col(pHAD28), Col3M

Serovars	Plasmid amplicons
S. Aberdeen	
S. Saintpaul	Col(pHAD28)
S. Chester	Col(pHAD28)
S. Mkamba	
S. Potsdam	
S. Tananarive /S. Brunei	IncFII(S)
S.Wa	

The comparison of plasmid regions and ARGs revealed that 11 *Salmonella* strains were carrying plasmids. Specifically, Col(pHAD28), IncX1, IncX2, Col3M, IncQ2, IncHI2, and IncHI2A were observed to harbor ARGs (Table 4). Of the 11 strains, only one, LH05301 (*S.* Corvallis), carried a resistance gene, *tet(A)*, located on the Col(pHAD28) plasmid within contig 36.

Table 4
Comparison of the plasmid replicons carrying antimicrobial resistance genes among *Salmonella* strains isolated from food samples in Cambodia.

Isolate	Serovars	Plasmidfinder		ARGs Analysis tools	
		Plasmids	Contig-Position	ARGs	Contig-Position
LH00501	S. Corvallis	Col(pHAD28) Col440I IncX2[a]	Contig25–4837..5210	qnrS1	Contig25–28111–28764
LH03601	S. Johannesburg	Col(pHAD28) Col3M[a]	Contig83–2130..2286	qnrD1	Contig83–1392–2036
LH04001	S. Agona	Col(pHAD28) IncX1[a]	Contig53–20794..21167	tet(A)	Contig53–12670–13866
LH04602	S. Heidelberg	Col3M	Contig61–2016..2172	qnrD1	Contig61–1278–1922
LH05101	S. Brancaster	Col(pHAD28) ColRNAI IncX1[a]	Contig21–5137..5510	blaTEM-176 aph(3')-Ia	Contig21–(150..1007) & (26080..26892)
LH05301	S. Corvallis	Col(pHAD28)[a] Col440I ColRNAI IncFIB(K)(pCAV1099-114)	Contig36–6677..6788	tet(A)	Contig36–6761-7957[b]
LH05601	S. Mbandaka	IncHI2[a] IncHI2A[a]	Contig11–166966..167292 Contig11–151469..152098	qnrS1 tet(A)	Contig11–1092-11745 Contig11–6403–7599
LH06003	S. Agona	IncX1[a]	Contig30–14527..14900	qnrS1	Contig30–1092–1745
LH06201	S. Corvallis	Col(pHAD28)[a]	Contig22–100..230	sul2	Contig22–757–1569
LH08801	S. Braenderup	Col440I IncQ2[a]	Contig59–148..597	qnrS2	Contig59–5497–6150

Isolate	Serovars	Plasmidfinder		ARGs Analysis tools	
		Plasmids	Contig-Position	ARGs	Contig-Position
LH13902	S. Molade / S. Wippra	Col(pHAD28)[a]	Contig24–68..198	qnrB19	Contig24–724–1365

[a] plasmid sharing the same contig as an antimicrobial resistance gene.

[b] plasmid and ARG are located on the same contig positions.

Salmonella virulent genes

According to the Virulence Factor Database (VFDB), 150 virulence genes with diverse mechanisms were identified (Supplemental Table 1). The majority of these genes functioned as Type Three Secretion System (TTSS) genes, fimbrial adherence genes, nonfimbrial adherence genes, macrophage-inducible genes, transporter genes, and magnesium uptake genes. Most TTSS genes were present across all *Salmonella* serovars, except *avr*A, which was absent in *S*. Livingstone, *S*. Indiana, *S.* Newport, and *S*. Mbandaka. Aside from TTSS, several virulence genes were identified across all serovars, including fimbrial adherence (*fim*CDFHI), nonfimbrial adherence (*sin*H, *mis*L), a macrophage-inducible gene (mig-14), a transporter gene (*csg*ABCDEFG, *fep*CG), and a magnesium uptake gene (*mgt*BC). Our results also showed that a few genes were found in specific serovars. For example, the *cdt*B gene, which encodes typhoid toxins, was identified in 7 serovars: *S*. Weltevreden, *S*. Indiana, *S*. Give, *S*. Johannesburg, *S*. Chester, *S*. Mkamba, and *S*. Potsdam. Gene *spv* encodes a plasmid virulence gene that was present in *S.* Typhimurium and *S.* Mbandaka, while the *pef*ABCD plasmid encoding fimbriae was found in *S.* Typhimurium, *S*. Weltevreden, and *S.* Mbandaka.

Moreover, 41 virulence genes were observed using the VFAnalyzer, such as *nlpI* (lipoprotein NlpI precursor), *shi*B (homologs of the *Shigella* flexneri SHI-2 pathogenicity island gene shiA), *fsr*B (biofilm formation), *fim*H (Type 1 fimbriae), *mrk*A (type 3 fimbrial major subunit), *terC* (Tellurium ion resistance protein), *tra*T (Outer membrane protein complement resistance), and *tcp*C (Tir domain-containing protein). Interestingly, *nlpI* was found in all *Salmonella* serovars. These results demonstrate that there were very few common virulence genes, such as *fim* and *tcp*C, which were identified by both VFAnalyzer and Abricate-VFDB.

Identification of Salmonella Pathogenicity Islands

In total, 12 *Salmonella* pathogenicity Islands (SPIs) were detected across 32 *Salmonella* serovars, namely C63PI, CS54_island, SPI-1 through SPI-5, SPI-8, SPI-9, SPI-12 to SPI-14 (Table 5). SPI-1 to SPI-3, SPI-5, and SPI-9 were present in all serovars, whereas SPI-12 was found exclusively in a single *S.* Braenderup. Moreover, 11 distinct SPI patterns were recorded, designated 1–11 (Table 5). The common serovars detected in this study displayed varying SPI profiles. For example, *S*. Corvallis and *S*. Agona exhibited SPI pattern 1; *S*. Weltevreden, pattern 2; *S*. Livingstone, pattern 7; and *S*. Braenderup, pattern 9.

Table 5
Salmonella pathogenic islands (SPIs) among 32 *Salmonella* serovar isolates from food products sold at local markets in Cambodia.

Pattern No.	SPI profiles	Serovars
Pattern 1	C63PI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-8, and SPI-9	*S*. Corvallis, *S*. Agona, *S*. Kentucky, and *S*. Brancaster
Pattern 2	C63PI, CS54_island, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, and SPI-14	*S*. Haifa, *S*. Typhimurium, *S*. Weltevreden, *S*. Bareilly, *S*. London, *S*. Heidelberg, *S*. Newport, and *S*. Aberdeen.
Pattern 3	C63PI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, and SPI-14	*S*. Uganda, *S*. Hvittingfoss, *S*. Mbandaka, *S*. Anatum, *S*. Give, *S*. Johannesburg, and *S*. Chester
Pattern 4	C63PI, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13, and SPI-14	*S*. Potsdam and *S*. Wa
Pattern 5	SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, and SPI-9	*S*. Molade / *S*. Wippra and *S*. Indiana
Pattern 6	C63PI, CS54_island, SPI-1, SPI-2, SPI-3, SPI-5, and SPI-9	*S*. Thompson and *S*. Tananarive /*S*. Brunei
Pattern 7	CS54_island, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, and SPI-14	*S*. Livingstone
Pattern 8	C63PI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-8, SPI-9, SPI-13, and SPI-14	*S*. Kedougou
Pattern 9	C63PI, CS54_island, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-12, SPI-13, and SPI-14	*S*. Braenderup
Pattern 10	CS54_island, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, and SPI-14	*S*. Saintpaul
Pattern 11	CS54_island, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, and SPI-14	*S*. Mkamba

Discussion

The presence of *Salmonella* spp. in fresh food produce has been extensively documented [6, 10, 26, 51]. In addition to its presence in various foods, numerous studies have also reported an increase in resistant strains, which raises public health concerns worldwide [36, 66].

This study revealed a potential serovar-food association, with *S.* Corvallis and *S.* Agona being the most frequently found serovars in meat products, *S.* Livingstone and *S.* Braenderup in seafood/fish, and *S.* Weltevreden in vegetables. *S.* Corvallis has been previously isolated from the informal market in Cambodia [63], and from chicken and pork in China [43, 45]. *S.* Agona has also been reported as a common serovar in raw meat products sold in small retail markets in China [72]. Indeed, *S.* Agona, *S.*

Livingstone, and *S.* Braenderup have also been reported in food chains worldwide, for example, in Europe, Australia, and Latin America [13, 22, 33, 53, 54]. Both *S.* Weltevreden and *S.* Typhimurium were detected in vegetables and seafood/fish. This aligns with studies from Vietnam and Malaysia, where *S.* Weltevreden was the predominant serovar in salad vegetables and water samples [24, 52]. Globally, *S.* Anatum and *S.* Weltevreden are commonly found in beef and seafood [21], while studies in China have noted the prevalence of *S.* Typhimurium and *S.* Weltevreden in retail meats [69]. These findings suggest that fresh food products in local markets may constitute the primary source of human salmonellosis.

The ARGs detected by the three analysis tools matched those that are most often detected. Similar to our results, several studies conducted in various countries have reported the predominance of these genes among *Salmonella* strains isolated from different food sources. In Latin America, *S.* Braenderup was mainly associated with the *qnrB19* gene, while *S.* Panama primarily carried *floR* and *tet(A)* genes [13]. In the Philippines, *qnrS* and bla_{TEM} were found in 61% of *Salmonella* isolates from pork, with 45.5% exhibiting co-carriage of bla_{TEM} and between one and three *qnr* subtypes [7]. In China, *qnr* genes were detected in 21% of isolates from chicken farms, slaughterhouses, and markets, with the highest frequency observed in markets (36%) [12]. Similarly, in the United States, *S.* Senftenberg strains from animal products harbored several resistance genes, including *aac*(6')-Iaa, *aph*(3")-Ib, *aph*(6)-Id, bla_{TEM-1}, *sul*1, *sul*2, and *tet*A [64]. In addition to the specific ARGs mentioned above, this study also detected common MDR genes, which the three tools identified as oqxA and oqxB. These genes encode resistance-nodulation-cell division (RND) antibiotic efflux pumps that confer resistance to various antibiotic classes, including fluoroquinolones, glycylcyclines, tetracyclines, diaminopyrimidines, and nitrofuran [4, 40]. This finding indicates a growing prevalence of MDR *Salmonella* strains throughout the Cambodian fresh food production chain.

Plasmids play a key role in the emergence and dissemination of AMR genes and other virulence factors among various *Salmonella* serovars [47, 59]. Our study revealed that most *Salmonella* serovars carried multiple plasmids, with the most common plasmids belonging to the Colistin (Col family) and Incompatibility (Inc) groups. Previous studies have reported that the Col(pHAD28) plasmid is associated with *qnr* genes conferring fluoroquinolone resistance, whereas IncC and IncQ1 plasmids are known to carry MDR genes [32, 49]. IncF plasmids, including IncFII(p96A), IncFII(S), and IncFIB(S), were identified in several serovars, such as *S.* Haifa, *S.* Typhimurium, *S.* Weltevreden, and *S.* Mbandaka. In contrast, IncH plasmids (IncHI2 and IncHI2A) were only found in *S.* Haifa and *S.* Mbandaka. In accordance with our findings, IncF plasmids are host-restricted and contribute to increased *Salmonella* virulence, whereas IncHI1 and IncHI2 are not host-restricted and are associated with resistance genes such as *tet*B, as well as genes conferring resistance to streptomycin and sulfonamides [47]. Detection of ARGs on plasmids showed that only one isolate, *S.* Corvallis, possessed a plasmid carrying a resistance gene, *tet*(A). Most ARGs, however, were not located on plasmid-associated contigs, suggesting that the majority of the identified ARGs were chromosomally encoded. Indeed, a study by Robertson et al. (2023) found that most *Salmonella* plasmids are not associated with ARGs. Although various plasmids were detected among *Salmonella* serovars, the limited occurrence of resistance gene-associated plasmids may

suggest that to effectively address AMR transmission in *Salmonella*, a greater focus should be placed on understanding the genomic context rather than plasmid-mediated mechanisms.

Salmonella WGS analysis revealed a high diversity of virulence genes; a total of 150 virulence genes were identified through ABRicate-VFDB, while 41 virulence genes were identified by VFAnalyzer. Similarly, a high diversity of virulence genes was reported in a study of MDR *Salmonella* isolates from poultry products, which identified 230 virulence genes in *S.* Enteritidis and 163 virulence genes in *S.* Infantis [34]. Most of the genes identified in this study were associated with infection, transmission, and proliferation in various hosts. These included TTSS genes, adherence genes, transporter genes, macrophage-inducible genes, endotoxin genes, and genes involved in magnesium uptake. Several WGS studies on *Salmonella* have documented virulent genes with functions similar to those identified in our study [17, 70]. A study on *Salmonella* 4,[5],12:i:- with a high AMR profile revealed a high diversity of virulence genes, such as *inv*A, *sse*L, *mgt*C, *si*iE, *sop*B, *gip*A, *gtg*B, *ssp*H1, and *ssp*H2, and most of these genes were identified across this study's serovars [44]. Plasmid virulence gene *spv* and plasmid-encoded fimbriae *pef*, both identified in *S*. Typhimurium, were also identified in similar studies [50, 15]. Moreover, the toxin and stress response genes identified in the current study were also reported in *Salmonella* isolates from Eastern and Southern Africa, such as *cdt*B and *mgt*B [48]. A report on *Salmonella* in Brazil identified 129 virulence genes, many of which are also common in this study, such as *mgt*C, *csg*C, *ssa*I, and *ssa*S [17]. This study also observed *that the csg*ABCD *genes were present in all Salmonella strains, whereas only 38* of 75 isolates possessed *csg*EFG. Interestingly, the study by dos-Santos et al. (2021) reported that 99% of *Salmonella* strains carried the *csg*ABCDEFG gene cluster, which is involved in fimbrial biosynthesis and contributes to enhanced biofilm formation, thereby promoting increased antibiotic resistance.

Both VFAnalyzer and Abricate-VFDB detected virulence genes, such as the fimbriae adherence gene (fim), which is present in *S*. Corvallis and *S*. Kedougou, and the Toll/interleukin-1 receptor domain-containing protein (tcpC), found in *S.* Anatum. *Salmonella* fimbriae play a crucial role in attaching to and invading the host epithelium. Among the fimbriae genes, *fim* is present in all strains of *S. enterica* but absent in *S.* bongori [58]. The *tcpC* gene encodes a protein homologous to human TIR domains and has been reported in *E.* coli to suppress the host's innate immune response, thereby enhancing bacterial survival [14, 68]. *S.* Enteritidis has been seen to possess *Tcp*S, a protein containing both a TIR domain and a human-like coiled-coil region, which functions as immune evasion [67].

The study on SPIs identified 12 SPIs across 32 *Salmonella* serovars, with SPI-12 found only in *S.* Braenderup. In South Korea, a study on MDR *Salmonella* isolates from poultry meat products exhibited 11 SPIs in *S.* Enteritidis and nine SPIs in *S.* Infantis [34]. In Nigeria, *Salmonella* serovars isolated from poultry have been reported to harbor both SPI-1 and SPI-2, which are essential for achieving their full virulence potential. Our findings aligned with this, as both SPI-1 and SPI-2 were present across the analyzed strains [32]. SPI-1 and SPI-2 encode *Salmonella* TTSS, which are related to the secretion of many important effector proteins. SPI-3 and SPI-4 are linked to long-term interactions and colonization in intestinal epithelial cells, whereas SPI-5 is involved in several stages of the infection process [48]. Additionally, eleven distinct SPI patterns were identified, each associated with a different *Salmonella*

serovar. Four unique SPI patterns were also reported among *Salmonella* enterica isolates from dogs and cats in Thailand [56]. Furthermore, a study on MDR *Salmonella* strains from retail meats and food animals in the United States demonstrated a high diversity of SPI profiles across various serovars [39].

Conclusion

The present study provided an in-depth understanding of the identified *Salmonella* serovars by characterizing their genomes, including ARGs, plasmids, virulence genes, and SPIs. Our findings revealed a high genetic diversity among *Salmonella* serovars, which may explain their ability to survive and disseminate through complex food matrices and along the food production chain in Cambodia. Furthermore, this study demonstrated that *Salmonella* isolates from meat, seafood/fish, and vegetables collected from local markets and farms in Cambodia share similar genetic traits. High gene expression, combined with the presence of virulence factors, allows these *Salmonella* strains to develop into highly virulent variants with differing capacities to cause infections in humans. Taken together, these findings underscore the urgent need for control strategies at both farm and market levels to prevent the transmission of highly virulent strains to humans.

Abbreviations

AMR Antimicrobial resistance

ARGs Antimicrobial-resistant genes

WGS Whole-genome sequencing

SPIs *Salmonella* pathogenicity islands

TTSS Type Three Secretion System

Declarations

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Author contributions

Laingshun Huoy: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Writing-original draft. **Kongkea Phan and Siteng Tieng**: Methodology, Writing-review & editing. **Leila Nasirzadeh:** Data curation, Formal analysis, Validation, Writing-review & editing. **Sofia Boqvist, Susanna Sternberg-Lewerin, and Erik Bongcam-Rudloff**: Conceptualization, Supervision, Writing-review & editing.

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Data availability

All raw sequence data from this project were deposited in the European Nucleotide Archive (ENA Accession * PRJEB110314*).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics, Consent to Participate, and Consent to Publish declarations

Not applicable.

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Figures

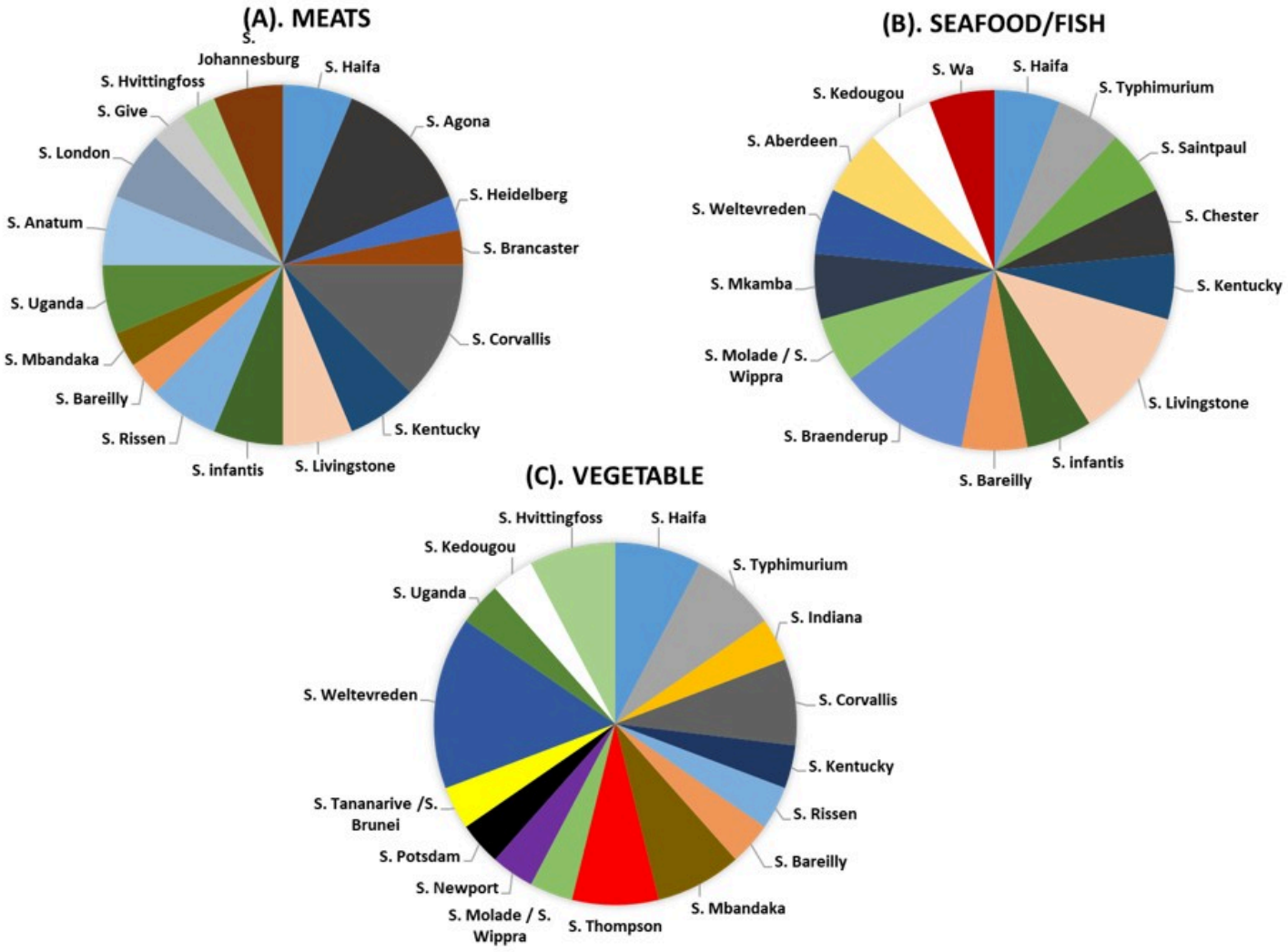


Figure 1

Salmonella serovars detected among food products sampled at local markets in Cambodia.

Supplementary Files

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- SupplementaryTable1.docx