

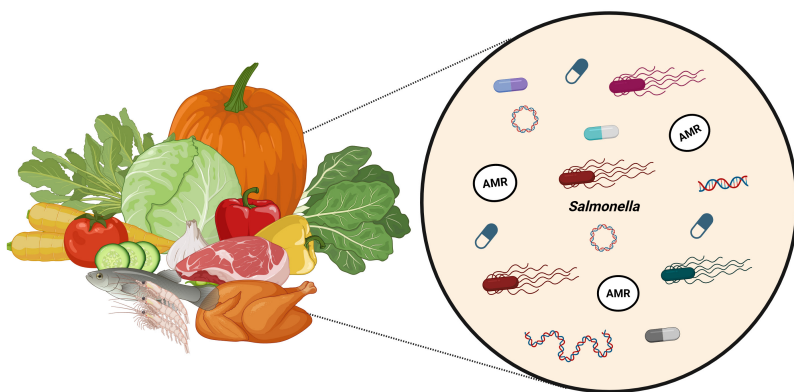


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Salmonella spp. isolated from foods in Cambodia:

Genetic characterization and antimicrobial resistance

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Genetic characterization and antimicrobial resistance

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Salmonella spp. isolated from foods in Cambodia: Genetic characterization and antimicrobial resistance

Abstract

Salmonella is a major global public health concern. In Cambodia, data on its prevalence in the food chain is limited, despite salmonellosis being a notable cause of gastrointestinal illness, particularly among children. This thesis aimed to generate new knowledge on the occurrence of *Salmonella* and its genetic traits associated with antimicrobial resistance (AMR) in fresh food products in Cambodia. A total of 285 food samples, including 75 meats, 50 seafood/fish, and 160 leafy green vegetables, were randomly collected from Phnom Penh local markets and nearby vegetable farms during 2020-2021. Cross-contamination risks were observed during food sampling through a checklist and a brief interview assessing hygiene practices and food handling procedures. As outlined in ISO 6579-1:2017, culture-based methods were employed to isolate *Salmonella* from all food samples, which were confirmed through biochemical, serological, and PCR tests. Following this, the Antimicrobial Susceptibility Test (AST) was conducted for the AMR phenotypes among *Salmonella* isolates. Whole-genome sequencing (WGS) analysis was performed to identify serovars and detect antimicrobial resistance genes (ARGs), plasmids, virulence genes, and *Salmonella* pathogenicity islands (SPIs). A high prevalence of *Salmonella* spp. 48% (138/285) was reported across samples, with the highest occurrence in meat (71%). Thirty-two distinct serovars were detected, with the most common being: *S. Corvallis*, *S. Haifa*, *S. Weltevreden*, *S. Agona*, *S. Kentucky*, and *S. Livingstone*. Inadequate hygiene and sanitation practices during handling, storage, and preservation at both farm and market levels, which likely cause cross-contamination opportunities, were also observed. The AMR result showed widespread resistance, affecting 71% of isolates, including 39% with multidrug resistance (MDR) supported by a diverse range of AMR-associated genes. High genetic diversity was detected, including plasmids, virulence-associated genes, and SPIs linked to AMR. These findings highlight the urgent need for integrated food safety interventions and robust antimicrobial stewardship to mitigate the risk of foodborne transmission of AMR *Salmonella* in Cambodia.

Keywords: *Salmonella*, prevalence, foodborne illness, food safety, local market, Phnom Penh.

Salmonella spp. isolerade från livsmedel i Kambodja: Genetisk karaktärisering och antimikrobiell resistens

Sammanfattning

Salmonella är ett stort globalt folkhälsopproblem. I Kambodja finns begränsade data om dess förekomst i livsmedelskedjan, även om salmonellos är en betydande orsak till mag-tarmsjukdomar, särskilt hos barn. Denna avhandling har genererat ny kunskap om förekomsten av *Salmonella* och dess genetiska egenskaper associerade med antimikrobiell resistens (AMR), i färska livsmedelsprodukter i Kambodja. Totalt provtogs 285 olika livsmedel, inklusive 75 prover från kött, 50 från skaldjur/fisk och 160 från bladgrönsaker, slumpmässigt från lokala marknader i Phnom Penh och närliggande gårdar under 2020–2021. Risker för korskontaminering dokumenterades vid provtagningen med hjälp av en checklista, och korta intervjuer med försäljarna genomfördes för att undersöka hygienrutiner och livsmedelshantering. Samtliga livsmedelsprover analyserades för *Salmonella* genom bakteriell odling enligt metoden ISO 6579-1:2017, och resultaten bekräftades genom biokemiska och serologiska analyser, samt med PCR. Därefter genomfördes antibiotikaresistens-tester för att avgöra AMR-fenotyper bland *Salmonella*-isolaten. Helgenomsekvensering (WGS) utfördes för att identifiera serovarer, samt för att identifiera AMR-gener, plasmider, virulensgener och *Salmonella* patogenicitetsöar. Resultaten visade att det var en generellt hög prevalens av *Salmonella* spp. (48%; 138/285) och förekomsten var högst i kött (71 %). Trettio två olika *Salmonella* serovarer identifierades och de vanligaste förekommande var: *S. Corvallis*, *S. Haifa*, *S. Weltevreden*, *S. Agona*, *S. Kentucky* och *S. Livingstone*. Ytterligare resultat visade på otillräcklig livsmedelshygien and bristande hantering, lagring och förvaring på både gårds- och marknadsnivå, vilket kan bidra till korskontaminering av *Salmonella*. AMR-undersökningen visade att 71 % av isolaten var resistenta mot minst ett antibiotikum och av dessa var 32 % multiresistenta. Hög genetisk diversitet påvisades, inklusive plasmider, virulensassocierade gener och *Salmonella*-patogenicitetsöar kopplade till AMR. Dessa resultat understryker det akuta behovet av att stödja åtgärder som främjar livsmedelssäkerhet och motverkar spridning av antibiotikaresistens, för att minska risken för livsmedelsburen AMR *Salmonella* i Kambodja.

Nyckelord: *Salmonella*, prevalens, livsmedelsburen sjukdom, livsmedelssäkerhet, lokal marknad, Phnom Penh

បាក់តេរី Salmonella ដែលញែកចេញពីអាហារស្រស់នៅកម្ពុជា៖ ការកំណត់លក្ខណៈហ្វេនូទែន និងភាពធន់នឹងថ្នាំប្រឆាំងមេរោគ

សេចក្តីសង្ខេប

បាក់តេរី *Salmonella* គឺជាត្រីបារម្ភចម្បងសម្រាប់សុខភាពសាធារណៈនៅទូទាំងពិភពលោក។ នៅកម្ពុជា ការសិក្សាអំពីវត្តមានរបស់វាក្នុងខ្សែច្រវាក់ចំណីអាហារនៅមានកម្រិត បើទោះបី ជាបាក់តេរីនេះ ជាភ្នាក់ងារចម្បងបង្កជំងឺគ្រុនពោះវៀន ជាពិសេសលើកុមារ។ ការសិក្សានេះមានគោលបំណង យល់ដឹងអំពីវត្តមានបាក់តេរី *Salmonella* និងលក្ខណៈដែលវាធន់នឹងថ្នាំប្រឆាំងមេរោគ (AMR) នៅក្នុងផលិតផលអាហារស្រស់នៅកម្ពុជា។ ភាគសំណាកអាហារសរុប ២៨៥ រួមមាន សាច់ ៧៥ គ្រឿងសមុទ្រ/ត្រី ៥០ និងបន្លែស្លឹកបៃតង ១៦០ ត្រូវបានប្រមូលពីផ្សារមូលដ្ឋាន និងកសិដ្ឋានបន្លែធានីក្រុងភ្នំពេញ ក្នុងកំឡុងពេលឆ្នាំ ២០២០-២០២១។ វិធីសាស្ត្រស្តង់ដារ ISO 6579-1:2017 ដោយផ្អែកលើថ្នាលបណ្តុះបាក់តេរីត្រូវបានប្រើ ដើម្បីញែក *Salmonella* ចេញពីគម្រូអាហារទាំងអស់ ហើយធ្វើការបញ្ជាក់តាមតេស្តគីមីដ៏រហ័ស សេរ៉ូមីឡូ និងប្រតិកម្មច្រវាក់ប៉ូលីមែរ៉ាស (PCR)។ បន្ទាប់មក ការធ្វើតេស្តភាពធន់នឹងថ្នាំប្រឆាំងមេរោគ (AST) ត្រូវបានអនុវត្តលើបាក់តេរីដែលញែកបានដើម្បីកំណត់លក្ខណៈ AMR។ ការវិភាគតាមតំណលំដាប់សេណូមទាំងមូល (WGS) ក៏ត្រូវបានអនុវត្ត ដើម្បីកំណត់ប្រភេទ serovar និងរកហ្វែនូទែនដែលទាក់ទងនឹងភាពធន់នៃថ្នាំ (ARGs) ប្លាស្ទីត ហ្វែនូទែនបង្កគ្រោះថ្នាក់ និងចំណុចបង្កជំងឺ (SPIs)។ លទ្ធផលបង្ហាញថា *Salmonella* មានវត្តមានខ្ពស់រហូតដល់ទៅ ៤៨% នៃសំណាកទាំងអស់ ដែលសាច់មានអត្រាខ្ពស់បំផុត (៧១%)។ ការរកឃើញ serovar ខុសៗ គ្នាចំនួន ៣២ ប្រភេទ ដែលប្រភេទរកឃើញភាគច្រើនរួមមាន៖ *S. Corvallis*, *S. Haifa*, *S. Weltevreden*, *S. Agona*, *S. Kentucky*, និង *S. Livingstone*។ លទ្ធផល AMR បង្ហាញពីភាពធន់ខ្ពស់រហូតដល់ ៧១% នៃបាក់តេរីដែលបានញែក ដែល ៣៩% មានភាពធន់នឹងថ្នាំច្រើនប្រភេទ (MDR)។ ការវិភាគហ្វែនូទែនបង្ហាញពីភាពចម្រុះខ្ពស់ រួមមាន ARGs ប្លាស្ទីត ហ្វែនូទែនបង្កគ្រោះថ្នាក់ និង SPIs ដែលអាចទាក់ទងនឹង AMR។ លទ្ធផលទាំងនេះបង្ហាញពីតម្រូវការបន្ទាន់សម្រាប់អន្តរាគមន៍សុវត្ថិភាពអាហារ និងការគ្រប់គ្រងការប្រើថ្នាំប្រឆាំងមេរោគរួមគ្នា ដើម្បីកាត់បន្ថយហានិភ័យនៃជំងឺ salmonellosis តាមរយៈអាហារនៅកម្ពុជា។

ពាក្យគន្លឹះ៖ *Salmonella*, អត្រាវិកលដាល, ជំងឺបង្កដោយអាហារ, សុវត្ថិភាពអាហារ, ផ្សារក្នុងស្រុក, ភ្នំពេញ។

Dedication

To my esteemed parents, my devoted husband and son, and my supportive brothers and sisters, whose unconditional encouragement has sustained me throughout my education journey.

The goal of education is the advancement of knowledge and the dissemination of truth—John F. Kennedy

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List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I. Huoy*, L., Vuth, S., Hoeng, H., Chheang, C., Yi, P., San, C., Chhim, P., Thorn, S., Ouch, B., Put, D., Aong, L., Phan, K., Nasirzadeh, L., Tieng, S., Bongcam-Rudloff, E., Sternberg-Lewerin, S., Boqvist, S. 2024. Prevalence of *Salmonella* spp. in meat, seafood, and leafy green vegetables from local markets and vegetable farms in Phnom Penh, Cambodia. Food Microbiology. 124, 104614.
<https://doi.org/10.1016/j.fm.2024.104614>
- II. Huoy*, L., Nasirzadeh, L., Phan, K., Tieng, S., Bongcam-Rudloff, E., Sternberg-Lewerin, S., Boqvist, S. 2025. Antimicrobial resistance and serotype distribution of *Salmonella* spp. isolated from fresh food in Cambodia. Journal of Applied Microbiology. 136(6):1xaf137. <https://doi.org/10.1093/jambio/1xaf137>
- III. Huoy*, L., Nasirzadeh, L., Phan, K., Tieng, S., Bongcam-Rudloff, E., Sternberg-Lewerin, S., Boqvist, S. Insights into Genetic Diversity of Cambodian *Salmonella* serovars via Whole-Genome Analysis. (Manuscript)

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The contribution of Laingshun Huoy to the papers included in this thesis was as follows:

- I. First author. Designed the whole study process. Managed and participated in the field sampling, laboratory performance, and data analysis. Main writer of the manuscript.
- II. First author. Designed the study. Conducted experiments (bacterial identification, antimicrobial susceptibility test, DNA extraction, and sample preparation for sequencing) and data analysis. Main writer of the manuscript.
- III. First author. Designed the study. Participated in laboratory performance (DNA extraction and sample preparation for sequencing). Partially performed data analysis and visualization. Main writer of the manuscript.

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Abbreviations

ADT	Agar Dilution Test
AMR	Antimicrobial Resistance
ARGs	Antimicrobial Resistance Genes
ASTs	Antimicrobial Susceptibility Tests
BDT	Broth Dilution Test
BHI	Brain Heart Infusion broth
BGA	Brilliant Green Agar
BPW	Buffer Peptone Water
CARD	Comprehensive Antibiotic Resistance Database
DDM	Disk Diffusion Method
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
FBD	Foodborne Diseases
FS	Fluorescence Spectroscopy
GST	Gradient Strip Test
IR	Infrared spectroscopy
LMIC	Low-and middle-income countries
LAMP	Loop-mediated isothermal amplification
MBC	Minimum Bactericidal Concentration
MDR	Multidrug-Resistance
MIC	Minimum Inhibitory Concentration
MKTTn	Muller-Kauffmann Tetrathionate Novobiocin broth
mPCR	Multiplex PCR
MS	Mass Spectrometry

NASBA	Nucleic Acid Sequence-based Amplification
NMR	Nuclear Magnetic Resonance
NTS	Non-typhoidal Salmonellosis
PCR	Polymerase Chain Reaction
qPCR	Quantitative real-time PCR
RM	Retail Market
RPA	Recombinase polymerase amplification
RS	Raman spectroscopy
RVS	Rappaport Vassiliadis Soya Peptone broth
RGI	Resistance Gene Identifier
SPIs	<i>Salmonella</i> Pathogenicity Islands
SS	<i>Salmonella-Shigella</i> agar
TS	Typhoidal Salmonellosis
TTSS	Type Three Secretion System
VF	Virulence Factor
VFDB	Virulence Factor Database
WGS	Whole Genome Sequence
WHO	World Health Organization
WM	Wholesale Market
XLD	Xylose-lysine-desoxycholate agar

1. Introduction

1.1 Introduction of foodborne diseases

1.1.1 Global burden of foodborne diseases

Foodborne diseases (FBD) represent a significant public health issue and constitute a considerable economic and social challenge worldwide. The World Health Organization (WHO) defines FBD as illnesses resulting from the consumption of food or water contaminated with harmful microorganisms or toxic substances, which leads to both morbidity and mortality in humans (Al-Mamun et al., 2018; Martín-Belloso & Elez-Martínez, 2005; WHO, 2022). Microorganisms responsible for FBD include harmful bacteria, viruses, and parasites. Bacterial pathogens are the most frequent cause and are often associated with contaminated food or water from the environment (Gupta, 2017). The main bacterial agents responsible for FBD are *Campylobacter* spp., *Salmonella* spp., *Escherichia coli*, *Yersinia* spp., and *Listeria* spp. (EFSA, 2025; Martín-Belloso & Elez-Martínez, 2005). A wide range of symptoms is caused by FBD-causing agents, which include fever, abdominal pain, nausea, vomiting, and diarrhea (Al-Mamun et al., 2018; Baird-Parker, 2016).

The true burden of FBD is difficult to assess due to underdiagnosis and underreporting. Moreover, food contamination is not the only route of transmission, which adds to the complexity of managing FBD. Nevertheless, many countries have made efforts to improve understanding of FBD incidence and its impact on both public health and the economy. The WHO recently reported that 31 foodborne hazards predominantly contribute to the global burden of FBD, causing around 600 million illnesses and 420,000 deaths each year due to unsafe food (Havelaar et al., 2015; WHO, 2022). Of these deaths, children under five years of age comprise 40%, accounting for about 125,000 cases annually. The leading causes of FBD are diarrheal disease-causing agents, which are responsible for at least 230,000 deaths globally. Of these, non-typhoidal *Salmonella enterica* is ranked as one of the top four causes, leading to an estimated 59,000 deaths each year (Havelaar et al., 2015). Additionally, reports from 27 countries within the European Union (EU) indicated that, in 2019, FBD-causing agents were responsible

for 49,463 illness cases, which resulted in 3,859 hospitalizations and 60 deaths (EFSA & ECDC, 2021; Sarno et al., 2021).

Beyond health impacts, FBDs impose a heavy economic and social burden that encompasses healthcare costs, productivity loss, and trade restrictions. The global burden of FBD is estimated at 33 million disability-adjusted life years (DALYs), with economic losses in low-income countries reaching approximately 110 billion USD each year (Havelaar et al., 2015; Jaffee, 2018; Nordhagen et al., 2022). Thus, many countries are now striving to better assess and address the burden of FBD to strengthen food safety systems and protect public health.

1.1.2 *Salmonella* epidemiology

Salmonella is a leading bacterial pathogen responsible for acute diarrheal illnesses, particularly in low- and middle-income countries (LMICs). Infection can occur through the ingestion of contaminated food or water, or through direct contact with contaminated animals and humans. The most common symptoms caused by this pathogen are gastroenteritis and enteric fever. It is categorized into two main types: typhoidal salmonellosis (TS) and non-typhoidal salmonellosis (NTS). TS is associated with *S. typhi* infection and presents symptoms such as fever, fatigue, and abdominal pain. NTS, caused by various *Salmonella* serotypes, typically manifests with fever, abdominal cramps, vomiting, and diarrhea (Crump et al., 2023; Crump & Wain, 2017; Naushad et al., 2023). Among them, *S. typhi* is the primary human-pathogenic strain that causes severe systemic illnesses, including fever, hepatomegaly, splenomegaly, and bacteremia (Lu et al., 2025; Zhao et al., 2025). TS serovars are host-restricted and transmitted between humans through contaminated food and water, whereas NTS serovars infect both humans and animals, and are primarily associated with acute gastroenteritis, which generally results in a limited systemic infection (Cheng et al., 2019; Lamichhane et al., 2024). Each year, foodborne *Salmonella* infections account for approximately 85% of all *Salmonella* cases globally, resulting in an estimated 93 million cases of gastroenteritis and approximately 155,000 deaths worldwide (Lamichhane et al., 2024). TS accounts for an estimated 11.9 million cases and 129,000 deaths in LMICs (Als et al., 2018). NTS is a major cause of bacterial foodborne infections, responsible for 3.4 million illnesses and 680,000 deaths annually, particularly within LMICs (Ajmera & Shabbir, 2023; Crump & Wain, 2017).

1.2 Overview of *Salmonella*

1.2.1 *Salmonella* taxonomy and classification

Salmonella is a genus of Gram-negative bacteria within the Enterobacteriaceae family (Issenhuth-Jeanjean et al., 2014; Popoff et al., 2004). These motile, facultative intracellular organisms are widely recognized as major foodborne enteric pathogens and are frequently linked to outbreaks of gastrointestinal illnesses (Knodler & Elfenbein, 2019). Recently, more than 2,600 *Salmonella* serotypes have been classified using the Kauffmann-White scheme (Achtman et al., 2012). These are divided into two species: *Salmonella bongori*, which predominantly infects cold-blooded animals, and *Salmonella enterica*, which is responsible for diseases in both humans and animals (Chattaway et al., 2021; Popoff et al., 2004). *S. bongori* was initially classified as subspecies V, comprising 22 serovars, whereas *S. enterica* is divided into six subspecies: enterica (I) with 1586 serovars, salamae (II) with 522 serovars, arizonae (IIIa) with 102 serovars, diarizonae (IIIb) with 308 serovars, houtenae (IV) with 76 serovars, and indica (VI) with 13 serovars (Achtman et al., 2012; Percival & Williams, 2014; Popoff & Le Minor, 2015; Ryan et al., 2017). *S. enterica* subsp. enterica is the primary cause of salmonellosis in humans (Lamas et al., 2018). *Salmonella* infections are classified into two main types: TS (i.e., *Salmonella* Typhi and *Salmonella* Paratyphi A) and NTS (e.g., *Salmonella* Enteritidis or *Salmonella* Typhimurium) (Oludairo et al., 2022). *Salmonella* serotypes are distinguished based on three surface structures: lipopolysaccharide (somatic O antigens), flagella proteins (H antigens), and capsular proteins (Vi antigens) (Brenner et al., 2000; Percival & Williams, 2014; Ryan et al., 2017). For instance, *Salmonella enterica* serotype I Typhimurium (*S. Typhimurium*) with antigenic formula 4,5,12:i:1,2 signifies that it belongs to subspecies I and possesses “4,5,12” O antigens, “i” as the phase 1 H antigen, and “1,2” as the phase 2 H antigen (Banerji et al., 2020; Grimont & Weill, 2007).

1.2.2 *Salmonella* morphology and basic microbiology features

Salmonella is a rod-shaped and non-spore-forming bacterium, typically measuring 2-5µm in length and 0.8-1.5µm in width. Although most *Salmonella* strains are motile due to the presence of peritrichous flagella, certain strains are non-motile, and these are usually found in clinical

environments (Percival & Williams, 2014). *Salmonella* is highly resilient in diverse environments, capable of persisting for several weeks under dry conditions, surviving for months in water, and remaining difficult to eliminate even in extremely cold environments (Naushad et al., 2023). Under suitable conditions, *Salmonella* can grow in both aerobic and anaerobic environments. Its optimal growth occurs at temperatures between 25 °C and 37 °C, and within a pH range of 6.0-8.0 (Keerthirathne et al., 2016; Nwabor et al., 2015). At present, commonly used selective cultures for screening and isolating *Salmonella* from food products include broth media such as Rappaport-Vassiliadis (RV), selenite or tetrathionate broths, and agar media such as Xylose Lysine Deoxycholate (XLD), Xylose-Lysine Tergitol (XLT), Deoxycholate Citrate (DCA), or Brilliant Green (BG) agar (Gorski, 2012; Neyaz et al., 2024; Percival & Williams, 2014). Colony morphology can often be distinguished visually using color indicators in each media type; for instance, typical *Salmonella* colonies grown on XLD agar appear pink or red with a black center (Neyaz et al., 2024).

1.2.3 *Salmonella* pathogenic mechanism

Most *Salmonella* strains are pathogenic, possessing the capacity to invade, colonize, and persist within host cells, which ultimately leads to diseases. The severity and type of infection vary depending on the specific serotype involved and the health status of the hosts (Eng et al., 2015; Li, 2022). Once *Salmonella* enters the host through ingestion of contaminated food or water, it employs multiple mechanisms such as adhesion, invasion, intracellular colonization, and localization within the host cell (Lu et al., 2025). Two key virulence components of type three secretion systems (TTSS), TTSS-1 and TTSS-2, play important roles in bacterial translocation. TTSS-1, encoded by *Salmonella* pathogenicity island 1 (SPI-1), facilitates bacterial invasion and translocation across the cell membrane, whereas TTSS-2, encoded by SPI-2, is associated with mechanisms that underlie systemic infections (Jiang et al., 2021; Kubori & Galán, 2002; Naushad et al., 2023). Other SPIs contribute to the bacterium's ability to adapt and survive within immune cells, such as macrophages, and encode the proteins that are involved in inflammation and the secretion of intestinal mucosal fluids (Eng et al., 2015; Lu et al., 2025; Ray et al., 2022). Additionally, various effector proteins, such as those encoded by virulence plasmids, along with fimbriae, flagella, and biofilm-associated proteins, also play key roles in *Salmonella* survival and

colonization within either the host or external environments (Quan et al., 2019; Silva et al., 2017; Upadhyay et al., 2025).

1.2.4 *Salmonella* sources and transmission route

Salmonella can spread through fecal contamination, and food may become contaminated during any stage of production, such as growing, harvesting, processing, storage, transport, or food preparation (Cheng et al., 2019). Foods often associated with *Salmonella* contamination include eggs, poultry, meat, dairy products, fresh seafood, fish, as well as raw fruits and vegetables (Al-Mamun et al., 2018). Eggs, egg products, and ready-to-eat foods are the primary sources of *Salmonella* outbreaks within the EU (De Cesare, 2018). Meat and poultry are acknowledged as primary reservoirs, while seafood, fish, vegetables, and dairy products are also important sources (Crump & Wain, 2017; Lamichhane et al., 2024).

Salmonella can persist for extended periods in the external environment, enabling it to survive in farm and feed products and facilitating its spread to agricultural fields as well as livestock-derived products (Andino & Hanning, 2015). The study by Andino et al. (2014) demonstrated that there are eleven *Salmonella enterica* serovars (Typhimurium, Enteritidis, Kentucky, Seftenberg, Heidelberg, Mbandaka, Newport, Bairely, Javiana, Montevideo, and Infantis) with this capacity in poultry feed. The two common serovars, *S. Typhimurium* and *S. Enteritidis*, both exhibit strong adaptability across diverse host environments, including human bodies, agricultural settings, livestock farms, and food industry environments (Campioni et al., 2018; Gantois et al., 2009; Martinez-Sanguiné et al., 2021; Perez-Sepulveda & Hinton, 2025). Further, these serovars frequently harbor virulence plasmids and other regulatory factors that contribute to antimicrobial resistance (AMR) mechanisms (Guillén et al., 2021).

1.3 *Salmonella* burden in Cambodia

1.3.1 Regional context of *Salmonella*

In Southeast Asia, foodborne salmonellosis is a significant concern due to rapid population growth, an increasing demand for animal-source foods, and limited resources for food safety regulation (Salvador et al., 2022; Talukder et al., 2023). Only a small number of studies have examined the incidence of

TS in Cambodia, with most focusing on the prevalence of *S. Typhi* and *S. Paratyphi A* together with their antimicrobial resistance (AMR) patterns (Emary et al., 2012; Kheng et al., 2020; Kuijpers et al., 2018; Pham Thanh et al., 2016; Wijedoru et al., 2012). A few studies have reported a high prevalence of *Salmonella* in food products, along with the frequent occurrence of both TS and NTS serotypes, as well as the AMR profiles (Chea et al., 2025; Chea et al., 2021; Salazar et al., 2025). However, although research studies on *Salmonella* in Cambodia have increased in recent years, comprehensive information regarding its prevalence and serotype distribution, as well as AMR of *Salmonella* in Cambodian food products, remains limited.

1.3.2 Market dynamics and foodborne *Salmonella*

Food safety continues to be a major challenge in LMIC countries, including Cambodia. There, both urban and rural communities are highly dependent on household food supplies purchased from local markets. Cambodian markets are organized into four administrative levels: provincial, district, commune, and village markets, which provide both food and non-food items (Duong et al., 2023; WFP, 2010). In addition, the country's main market types have recently begun to consist more of wholesale markets, retail markets, and supermarkets/superstores (CDC & Cambodia, 2013). Aside from supermarkets/superstores, most local Cambodian markets operate in open areas, where both stationary and mobile vendors are present. These markets often lack food safety standard regulation, and frequently use improper storage temperatures, along with unclean water for rinsing or moistening vegetables to maintain freshness, resulting in the introduction and spread of foodborne pathogens across food products (Huoy et al., 2024; Mosimann et al., 2023; WHO, 2024).

Cambodian local markets, characterized by inadequate hygiene, sanitation, and infrastructure, are likely associated with the growing risk of foodborne pathogen contamination. Studies show that fresh food products (fresh vegetables and meats) in local markets have a high prevalence of microbial contamination, including *Salmonella enterica* (42-88%), *S. aureus* (29.1%), *E. coli* (9-34%), and *Campylobacter* spp. (56-81%) (Chhim et al., 2022; Desiree et al., 2021; Lay et al., 2011; Osbjør et al., 2016; Chea et al., 2021). However, fresh food products are not the only source of contamination. A study on fermented vegetables sold in local markets also showed high

contamination of *Enterococcus* spp. (34%) followed by *Bacillus* spp. (31%), coliform (24%), and *E. coli* (10%) (Chrun et al., 2017). Additionally, up to 75% of food contact surfaces were reported to be contaminated with *Salmonella* spp. (Schwan et al., 2021).

1.4 Risk factors for *Salmonella* contamination

In LMIC countries, *Salmonella* is highly prevalent in both food and environmental sources, accompanied by a significant number of human salmonellosis cases. The key risk factors for the persistence of *Salmonella* in food chains are linked with farm practices, food processing (including initial processing, handling, storage, and preservation), market practices, and risks related to cross-contamination (Asakura et al., 2023; Chea et al., 2025; Lindahl et al., 2020; Chea et al., 2022; Ström et al., 2018; Thompson et al., 2021).

At the farm level, poor biosecurity, contaminated feed, and close contact between animals facilitate disease transmission (Lindahl et al., 2020). A study on *Salmonella* incidence on Ethiopian poultry farms highlighted key risk factors driving its high prevalence and associated AMR, which included large flock sizes, unidentified poultry sources, poor management practices, and inadequate farm hygiene (Basazinew et al., 2025). Moreover, a study in China found that poor farm and biosecurity management, such as a lack of quarantine for new animals and insufficient feces removal (less than 50%), were major risk factors for *Salmonella* contamination on dairy cattle farms (Wang et al., 2023). In Cambodia specifically, the major risk factors associated with foodborne salmonellosis include the use of wastewater in agricultural production, poor slaughterhouse hygiene, and climate change/severe weather conditions, together with the limited knowledge and awareness of foodborne illnesses among farmers (Asakura et al., 2023; Chea et al., 2025). The detection of *Salmonella enterica* in manure samples collected from urban livestock keepers in Cambodia indicates that inadequate manure management practices, often without proper pre-treatment, are frequently employed. This poses a public health risk and underscores the need to improve livestock manure quality to prevent pathogen transmission to agricultural products (Ström et al., 2018).

The market environment also plays an important role in the transmission of foodborne pathogens. In LMIC countries, local markets are often informal

and open-air, with numerous vendors lacking food safety knowledge, which results in, for example, improper storage conditions, such as inadequate temperature control (Nidaullah et al., 2017; Schwan et al., 2021). A study on traditional markets in Surabaya, Indonesia, noted poor environmental conditions and improper food handling as key risk factors contributing to the high NTS contamination in chicken meat (Wibisono et al., 2023). In Cambodia, where wet markets and informal food vending are common, the risk of contamination is high because fresh food products are frequently stored under improper conditions; for instance, meat and seafood are often displayed without refrigeration, which creates favorable conditions for bacterial growth and spread (Chea et al., 2022).

At the household level, during food processing and storage, contamination risks are heightened by inadequate hygiene practices, poor temperature control, and insufficient sanitation (Ehuwa et al., 2021). A food safety study performed in Cambodia emphasized the risk factors linked to safe vegetable handling, including basic hygiene, handwashing prior to handling produce, proper washing methods, and the use of gloves to reduce cross-contamination (Thompson et al., 2021). Studies have shown that *Salmonella* grows more rapidly at high temperatures, which is associated with increased outbreak risks in environments lacking temperature control (Billah & Rahman, 2024).

Cross-contamination can occur at multiple stages of the food chain, from farms and processing plants to markets and household kitchens (Chapman & Gunter, 2018). For instance, *Salmonella* cross-contamination and recontamination in food are linked to poor sanitation practices, poor equipment design, and insufficient control of food ingredients (Carrasco et al., 2012; Podolak et al., 2010). Indeed, cross-contamination of *Salmonella* from raw meat to ready-to-eat foods has been associated with raw ingredient handling (meat and vegetables), the use of shared or unclean food processing tools (e.g., cutting boards, knives, and containers), and even cooking ingredients such as salt, which can act as effective pathogen carriers (Alves et al., 2022; Dantas et al., 2018; Chea et al., 2022).

1.5 Serovars of public health importance

Certain *Salmonella* serovars are more frequently associated with human illnesses and outbreaks than others. According to the EFSA and ECDC

(2021), the five most common serovars linked to human *Salmonella* in the EU/EEA are: *S. Enteritidis*, *S. Typhimurium*, *Salmonella* monophasic Typhimurium, *S. Infantis*, and *S. Derby*. In a study conducted between 2019 and 2021 in Korea, the predominant serovars identified in diarrheal patients, both domestic residents and returning travelers, were *S. Enteritidis*, *S. Typhimurium*, and its monophasic variant *S. 4,5,12:i:-* (Jeong et al., 2022). Moreover, a report containing data from 2004 to 2022 in Taiwan showed that the top five serovars across human infections were *S. Enteritidis*, *S. Typhimurium*, *S. Newport*, *S. Stanley*, and *S. Anatum* (Liao et al., 2024). In Cambodia, two common causes of typhoid salmonellosis, *S. Typhi* and *S. Paratyphi A*, have been reported among Cambodian children (Chheng et al., 2013; Emary et al., 2012; Kheng et al., 2020; Kuijpers et al., 2017; Vlieghe et al., 2013; Wijedoru et al., 2012).

Serovars such as *S. Enteritidis* and *S. Typhimurium* are common worldwide, while in Southeast Asia, other serovars, including *S. Weltevreden* and *S. Kentucky*, are becoming increasingly documented. A meta-analysis by Ferrari et al. (2019) showed that the distribution of common *Salmonella* serovars varied according to the type of food. In pork samples, *S. Typhimurium* and *S. Derby* (reported in the EU, Asia, Oceania, and North America), *S. Hadar* (Africa), and *S. Meleagridis* (Latin America) were prevalent. For poultry, frequent serovars include *S. Enteritidis* (Asia, Latin America, the EU, and Africa), along with *S. Kentucky*, *S. Typhimurium*, and *S. Sofia*, which have been reported in North America and Oceania. The dominant serovars in beef are *S. Anatum* and *S. Typhimurium* (Africa, Latin America, and the EU), while *S. Agona* is mostly reported in Asia. Regarding seafood, common serovars include *S. Hadar* (Latin America and Africa), *S. Typhimurium* (EU), *S. Weltevreden* (Asia), and *S. Newport* (North America) (Ferrari et al., 2019). In South Asia, a 10-year study investigating the prevalence and distribution of *Salmonella* serovars across human, animal, and environmental samples identified 18 serovars, among which *S. enterica* and *S. Pullorum* were the most prevalent, with animals serving as the primary source (Talukder et al., 2023). In addition to the serovars noted above, other serovars have been recorded, for example, *S. Rissen* is being reported as the most common serovar in meat products in Thailand and China (Patchanee et al., 2016; X. Xu et al., 2020). In Cambodia, several studies have found a high prevalence of *Salmonella* among food products and environmental sources (Desiree et al., 2021; Lay et al., 2011; Schwan et al., 2022; Ström et al.,

2018). Several serovars were identified, including *S. Rissen*, *S. Hvittingfoss*, *S. Corvallis*, *S. Krefeld*, *S. Weltevreden*, and *S. Altona* from food contact and non-food contact surfaces in Cambodian informal markets (Schwan et al., 2021). In another study in Cambodia, *S. Rissen* and *S. Anatum* were the most predominant serovars isolated from pig and pork samples (Lay et al., 2021).

Based on the above data, identifying the serovar distribution in local food products is crucial to understanding transmission dynamics and prioritizing public health interventions to control the spread of infection.

1.6 Antimicrobial Resistance (AMR) in *Salmonella*

1.6.1 General information on antibiotics and their mode of action

Antibiotics have been used extensively across the globe to both treat and prevent bacterial infections, and they have thus helped to lower mortality and morbidity rates for both humans and animals (Ahmed et al., 2024; Darby et al., 2023; Z. Fatima et al., 2023; Punchihewage-Don et al., 2024; Salam et al., 2023). Most antibiotics are produced naturally by microorganisms, but some are derived from synthetic substances, each with different abilities of inhibiting or eliminating harmful organisms (Hutchings et al., 2019; Pancu et al., 2021). They have long been used to treat bacterial infections in humans, animals, and crops, and their application has expanded to include growth promotion in livestock (Halawa et al., 2024). Antibiotics' mechanisms of action are classified into five major mechanisms: inhibiting the bacterial cell wall synthesis, inhibiting protein biosynthesis, inhibiting nucleic acid synthesis, inhibiting metabolic pathways, and inhibiting bacterial membrane function (Uddin et al., 2021). Antibiotics are categorized into several classes, namely β -lactams, quinolones, chloramphenicol, aminoglycosides, sulfonamides, and tetracyclines. Each class functions through distinct action mechanisms, and bacteria can develop resistance to them via various pathways (Punchihewage-Don et al., 2024).

β -lactam antibiotics, such as penicillin, ampicillin, and cephalosporin, act by inhibiting bacterial cell wall synthesis by binding to penicillin-binding proteins (PBPs), thereby blocking the final stage of peptidoglycan layer formation, which leads to loss of viability and cell lysis (Uddin et al., 2021). Quinolone antibiotics, such as ciprofloxacin and ofloxacin, interfere with

bacterial nucleic acid synthesis by blocking DNA replication or transcription (González-Villarreal et al., 2022; Pham et al., 2019). Phenicol, including Chloramphenicol, inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit (Roberts & Schwarz, 2016; Schwarz et al., 2016). Aminoglycoside antibiotics, such as gentamycin, streptomycin, and neomycin, function by binding to the A-site of the 16S rRNA within the 30S ribosome subunit, which leads to codon misreading and subsequent inhibition of protein synthesis (Krause et al., 2016; Punchihewage-Don et al., 2024). Sulphonamide antibiotics, including sulfamethoxazole or sulfonamide, inhibit folic acid synthesis, which is vital for DNA production in bacteria (Ovung & Bhattacharyya, 2021; Punchihewage-Don et al., 2024). The combination of trimethoprim-sulfonamide can block the synthesis of tetrahydrofolate and prevent DNA replication. Tetracycline antibiotics, including tetracycline and doxycycline, inhibit protein synthesis by blocking aminoacyl-tRNA from binding to the bacterial ribosome (Chopra & Roberts, 2001; Roberts & Schwarz, 2016). Lastly, macrolide antibiotics, such as erythromycin and azithromycin, are a hydrophobic antibiotic class that inhibit bacterial growth by binding to the 50S ribosome subunit, which blocks polypeptide formation and protein biosynthesis (Dinos, 2017; Nor Amdan et al., 2024).

1.6.2 Antimicrobial resistance mechanism

AMR has emerged as a critical challenge in managing *Salmonella* infections. The misuse and overuse of antibiotics in both human medicine and animal production aid in to the rise of resistant strains, as well as an increase in multidrug resistance (MDR) in microbes (Punchihewage-Don et al., 2024). MDR refers to the ability of an organism to resist the effects of three or more antimicrobial agents (Hassall et al., 2024). Bacteria can develop antibiotic resistance through chromosomal mutations or by obtaining resistance genes via horizontal gene transfer (HGT) or plasmid exchange between organisms (Belay et al., 2024; Z. Fatima et al., 2023; Salam et al., 2023). Resistance can arise through intrinsic, acquired, or adaptive mechanisms.

Intrinsic resistance refers to the natural ability of the organism to express chromosomal genes that provide protection against specific antibiotics (González-Villarreal et al., 2022). Common mechanisms consist of the action of efflux pumps and decreased membrane permeability, which restrict the entry of antibiotics into bacterial cells. One of the most common AMR

mechanisms is the use of efflux pumps, which expel antibiotics from the cell and consequently aid in the development of resistance (Belay et al., 2024). Efflux pumps are classified into two main types: primary transporters, which belong to the ATP-binding cassette (ABC) family, and secondary transporters, which include the major facilitator superfamily (MFS), resistance nodulation division (RND), small multidrug resistance (SMR), multidrug and toxic compound extrusion (MATE) family, and drug metabolite transporter (DMT) superfamily. A review of several studies on *Salmonella* efflux pumps was recently reported, and these included *AcrAB*, *AcrEF*, *AcrD*, *MdsABC*, *MdtABC*, *EmrAB*, *MdfA*, *MacAB*, and *MdtK* (Zhou et al., 2023). Furthermore, reduced membrane permeability involves two mechanisms: alterations of the outer membrane lipid barrier and porin-mediated permeability (Punchihewage-Don et al., 2024). For instance, *Salmonella* can reduce the negative charge on its cell surface by adding a positive charge to the lipid A, thereby lowering the affinity of antimicrobial peptides.

Acquired resistance arises from mutations or the acquisition of new genetic material through plasmid transfer and other mechanisms (e.g. transformation and transduction) (Uddin et al., 2021; Zhou et al., 2023). Alterations in nucleic acid strands, through either point mutation or multiple mutations, can possibly lead to resistance mechanisms that render antibiotics ineffective against microbes. In *Salmonella*, point mutations in the *gyrA* gene, which encode the gyrase subunit, confer resistance to quinolones, while alterations in protein channels *OmpC* and *OmpF* contribute to resistance; for example, the loss of *OmpC* results in *S. typhimurium* resistance to cephalosporins (Zhou et al., 2023). AMR genes may be located on chromosomes or plasmids, with plasmid-borne genes facilitating the spread of AMR within microbial populations (Belay et al., 2024; Naveed et al., 2020). For instance, *S. Heidelberg* acquired an IncK2 plasmid carrying the extended-spectrum- β -lactamase gene (*bla* CMY-2) after in vitro culture, likely through plasmid-mediated gene transfer. Another study on azithromycin resistance revealed that the *mph(A)* gene, located on various plasmids such as IncFIB, IncHI2, IncFII, IncC, and IncI carrying *intI1*, is indicative of MDR transmission among *Salmonella* strains (Zhang et al., 2024).

Adaptive resistance, in contrast, occurs when environmental factors, such as temperature, pH, growth conditions, nutrient availability, or stress, cause bacteria to resist antibiotics (Belay et al., 2024; Zhou et al., 2023). The

studies noted that biofilm formation is one of the most common strategies that microbes use to support cell survival, serving to establish infections (Ahmed et al., 2024; Belay et al., 2024; Darby et al., 2023). The common adaptive resistance in *Salmonella* includes tolerant cells, persistent cells, and biofilms. A recent report found that 2-5% of typhoid patients were asymptomatic carriers due to *Salmonella* forming biofilms (Zhou et al., 2023).

Although antibiotics can inhibit and kill microbes, organisms can develop defense mechanisms against these agents. For example, bacteria develop resistance by preventing antibiotics from interacting with the PBP, using efflux pumps to expel β -lactams, or producing β -lactamase enzymes that degrade these antibiotics. Common resistance genes include *bla*_{TEM-1}, *bla*_{TEM-20}, *bla*_{TEM-152}, *bla*_{CTX-M-1}, *bla*_{CMY-2}, *bla*_{OXA-1}, and *bla*_{PE-1}, which are present among *Salmonella* strains (Punchihewage-Don et al., 2024). A study also reported that *Salmonella* can become resistant to quinolone antibiotics through mutations in the quinolone resistance-determining region (QRDR) of *GyrA*, *GyrB*, *parC*, and *parE*, as well as modifications to the antibiotic target region. The major resistance genes are genes *gyrA*, *gyrB*, *parC*, *parE*, *qnrB*, *qnrD*, *qnrS*, and *oqxAB* (Punchihewage-Don et al., 2024).

In *Salmonella*, mechanisms for resistance against phenicols include efflux pump expression and enzymatic inactivation of the antibiotics. Resistance genes identified in the *Salmonella* isolate include *catA1*, *floR*, and *cmlA1* (Punchihewage-Don et al., 2024). Aminoglycoside antibiotics, such as gentamycin, streptomycin, and neomycin, function by binding to the A-site of the 16S rRNA within the 30S ribosome subunit, which results in codon misreading and subsequent inhibition of protein synthesis (Krause et al., 2016; Punchihewage-Don et al., 2024). The resistance mechanism can take various forms, such as enzymatic drug modification, target site modification, and the efflux-mediated mechanism (Krause et al., 2016). The common aminoglycoside resistance genes found among *Salmonella* isolates are *armA*, *rmtC*, *aadA1*, *aadA2*, *aadA5*, *aphA1AB*, *aac(3)-IV* *aph(3')-IIa*, *aacC2*, *aac(3)-Iva*, *aacA4*, *strA*, *strB*, *aadA*, *aphA2*, and *aphA1* (Punchihewage-Don et al., 2024). For trimethoprim-sulfamethoxazole, there are two main mechanisms used by bacteria to resist this antibiotic, and these primarily involve the mutation of the *folP* gene encoding dihydropteroate synthase (DHPS) and the acquisition of an alternative *DHPS* gene, causing low affinity to sulfonamides. The major resistance genes include

sulfonamide (*sul1*, *sul2*, and *sul3*) and trimethoprim (*dfrA* genes) (Antunes et al., 2005). Moreover, *Salmonella* often resists tetracycline antibiotics through mechanisms such as efflux pumps, modification of the tRNA target site, and inactivation of the antibiotic compound. The common resistance genes are *tetA*, *tetB*, *tetC*, *tetD*, and *tetG* (Punchihewage-Don et al., 2024). For macrolide antibiotics, bacterial resistance primarily occurs through target modification in the 23S rRNA binding site, while additional mechanisms include reducing intracellular antibiotic concentration and protecting the ribosome by altering its molecular conformation (Nor Amdan et al., 2024). Indeed, several macrolide resistance genes have been recently identified, such as *mef* genes (*mef(A)*, *mef(B)*, *mef(E)*, *mef(I)*, and *mef(O)*), *msr*, and *erm* genes (*ermA*, *ermB*, *ermC*, *ermD*, etc) (Dinos, 2017).

1.6.3 Role of food chains in spreading AMR

The rise of AMR and MDR represents a serious threat to human health. In Cambodia and other LMIC countries, MDR *Salmonella* not only complicates treatment but also poses additional risks of resistance gene transfer to other pathogens. AMR can spread through the food chain or among animals carrying resistant pathogen strains (Lin et al., 2021; Sagar et al., 2023). Food chains are acknowledged as an important route for AMR transmission, largely because of the indiscriminate use of antibiotics in crop cultivation and livestock production (Halawa et al., 2024). In crop cultivation, AMR/MDR bacteria can be transmitted through contaminated irrigation water and untreated animal manure that is used as fertilizer, with the spread occurring from the production stage to the markets and then ultimately to consumers (Z. Fatima et al., 2023). Across livestock production, the extensive use of antibiotics in animal feed for disease prevention and treatment promotes the spread and circulation of AMR among animal species and contributes to environmental contamination through fecal waste from infected animals (Ahmed et al., 2024).

Several studies have reported on the prevalence of *Salmonella* and its AMR within human, food, and environmental sources. Studies in Thailand and China have revealed that *S. Rissen* isolates from food products exhibit high resistance to ampicillin, tetracycline, and streptomycin (Patchanee et al., 2016; X. Xu et al., 2020; Z. Xu et al., 2020). The study conducted on fish and meat showed that the specific *Salmonella* serotypes isolated from the samples *S. Saintpaul* and *S. Newport*, *S. Rissen* and *S. Typhimurium*

4,5,12:i:-, were identified as ESBL-producing *Salmonella enterica*, which are resistant to almost all antibiotics used for severe salmonellosis treatment (Nadimpalli et al., 2019). Among NTS isolates from Cambodian diarrhoea patients, resistance to nalidixic acid, ciprofloxacin, and ampicillin was observed (Poramathikul et al., 2021). The growth of AMR in Cambodia is unsurprising, as limited antimicrobial stewardship and easy access to antibiotics without requiring a prescription have intensified the problem, raising concerns about the role of food chains in spreading resistant strains.

1.7 *Salmonella* spp. detection and identification methods

1.7.1 Conventional culture and biochemical methods

Traditional methods used to isolate *Salmonella* include culture-based techniques followed by biochemical and serological tests (Chaudhary et al., 2024; Patel et al., 2024). Culture-based methods are low-cost, highly sensitive, and can determine the viable cell count across various sample types. However, they involve multiple steps, including pre-enrichment, enrichment, isolation, and biochemical confirmation steps (ISO 6579-1:2017) (Guyassa & Dima, 2022; Oslan et al., 2024). The pre-enrichment step is the initial stage following sample processing, where the sample is homogenized in non-selective broths and incubated for 6 to 24 hours at 37 °C. Non-selective broths often used are buffered peptone water (BPW), nutrient broth (NB), tryptic soy broth (TSB), lactose broth (LB), and skim milk. The enrichment step employs selective media and requires 24 hours of incubation at varying temperatures. Selective enrichment broths include Rappaport-Vassiliadis (RV) broth, with an incubation temperature of 42 °C, selenite cystine (SC), and Muller-Kauffmann tetrathionate (MKTn) broth, at an incubation temperature of 37 °C. The isolation step is then carried out by spreading or streaking the enrichment culture onto selective agar plates, such as Xylose Lysine Deoxycholate (XLD), Xylose-Lysine Tergitol (XLT), Deoxycholate Citrate (DCA), or Brilliant Green (BG) agar. After 24 hours of incubation at 37 °C, characteristic *Salmonella* colonies can be observed, and the presumptive colonies are further confirmed through biochemical testing. The current biochemical assays include catalase, oxidase, oxidation-fermentation, indole, citrate utilization, triple sugar iron agar, and the urea hydrolysis test (Chaudhary et al., 2024; Guyassa & Dima, 2022). These

assays provide additional supporting information to verify and validate the culture result. Additionally, to enhance the reliability of culture and biochemical results, serological testing, also known as antibody-based agglutination, is performed based on bacterial cell surface characteristics. This method employs antisera targeting *Salmonella* surface antigens, including somatic (O), flagellar (H), and Vi antigens, to enable serogrouping and serotyping identification (Oslan et al., 2024).

1.7.2 Molecular methods

The advancements in molecular technologies throughout recent years have improved the efficiency of detecting foodborne *Salmonella*. These methods rely on identifying pathogen-specific genetic markers within complex environments. A variety of molecular techniques are available, such as polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), recombinase polymerase amplification (RPA), DNA microarray, and whole-genome sequencing (WGS) (Awang et al., 2021; Yang et al., 2025).

PCR is widely regarded as the gold standard for bacterial identification, including *Salmonella*. This method can synthesize thousands or even millions of copies of target RNA or DNA fragments from a single nucleic acid fragment based on three main steps: denaturation, annealing, and extension (Deb et al., 2024). By targeting the *invA* gene, this method enables rapid and precise detection, offering superior sensitivity and specificity compared with conventional culture-based techniques (Chirambo et al., 2020; Thung et al., 2019). Multiplex PCR (mPCR) is an advancement of conventional PCR, designed to amplify multiple target DNA sequences (or primers) simultaneously, detect various *Salmonella* serovars, or detect a combination of *Salmonella* and other foodborne pathogens within a single sample (Awang et al., 2021; Deb et al., 2024). Furthermore, quantitative real-time PCR (qPCR) is an advanced form of PCR that provides rapid and highly precise results, which can detect low concentrations of *Salmonella* without a pre-enrichment step. This method has been applied to identify *Salmonella* in various types of samples, including human, food, feed, and environmental sources (Chan et al., 2023; Chheng et al., 2013; Kasturi & Drgon, 2017; Malorny et al., 2008; Moore et al., 2014; Yang et al., 2021).

Another widely used molecular approach is WGS together with comparative analyses, and this method has received considerable attention throughout the

past decade (Elhassan et al., 2023; Ong Kar et al., 2021; Wan Makhtar et al., 2021). WGS involves sequencing the entire genome of the target microorganism, combined with reliable analysis tools and a reference database, which enables precise tracking of the transmission pathways of specific pathogenic species (Awang et al., 2021; Oslan et al., 2024). More recently, WGS has proved a powerful tool for accurate serotyping, antimicrobial resistance gene (ARGs) detection, and tracking transmission, allowing an action plan to be generated in *S. enterica* control (Banerji et al., 2020; Patra et al., 2025; Piras et al., 2021; Song et al., 2025). Applications of WGS analysis tools for *Salmonella* often include serotype prediction (SeqSero and SISTR), identification of antimicrobial resistance genes (ResFinder, AMRFinderPlus, ABRicate, and CARD-RGI), plasmid detection (PlasmidFinder), characterization of *Salmonella* pathogenicity Islands (SPIFinder), and analysis of virulence factors (VFAnalyzer and Virulence Factor Database (VFDB)) (Ibrahim & Morin, 2018; Patra et al., 2025; Pornsukarom et al., 2018; Robertson et al., 2018; Vakili et al., 2025). For instance, tools such as SeqSero2 enable reliable prediction of serotypes from genomic data, providing deeper insights into pathogen diversity and epidemiology (Zhang et al., 2019). Although WGS generates highly reliable data, its processing continues to present challenges due to limitations in available tools and pipelines, as well as the considerable costs associated with sequencing and analysis.

Both traditional and molecular methods are highly sensitive for detecting and characterizing *Salmonella*; however, traditional approaches are time-consuming and labor-intensive, while molecular techniques demand greater expertise and higher investments (Patel et al., 2024; Yang et al., 2025). To overcome the time-consuming nature of traditional and molecular methods, several commercial rapid techniques have been developed, offering reliable results at affordable operational costs. At present, commercially available approaches for *Salmonella* detection include selective media, immunology-based assays, and nucleic acid-based assays (Lee et al., 2015).

1.8 AMR identification methods

The increasing prevalence of AMR *Salmonella* across LMIC countries marks a significant public health challenge for infectious disease management. Various methods have been applied to study and characterize AMR

development, ranging from conventional approaches such as antimicrobial susceptibility test (AST) to advanced techniques including mass spectrometry (MS), infrared spectroscopy (IR), Raman spectroscopy (RS), fluorescence spectroscopy (FS), and nuclear magnetic resonance (NMR) (Ramzan et al., 2024). Advanced microscopic and spectroscopic methods (MS, IR, RS, and FS) have demonstrated potential as rapid and accurate diagnostic tools, offering valuable benefits for treating AMR-related infections (Ramzan et al., 2024). Nonetheless, conventional AST remains the most widely used approach, particularly in LMICs, as it allows for both evaluation of treatment effectiveness and prediction of therapeutic outcomes for specific antibiotics (Amin et al., 2022).

Conventional or traditional methods refer to the use of culture-based techniques, including broth dilution test (BDT), agar dilution test (ADT), disk diffusion method (DDM), and gradient strip test (GST) (Hassall et al., 2024; Kannan et al., 2024; Ramzan et al., 2024). BDT is a liquid culture-based method used to evaluate the sensitivity and resistance of microorganisms to specific antimicrobial agents. This test includes the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) test. ADT involves applying bacterial isolates onto agar media containing antibiotics, followed by visual observation of the colony's growth and its morphology. Moreover, the DDM, also known as the Kirby-Bauer test, is used to evaluate bacterial resistance to specific antibiotics using standardized commercial disks. The method relies on the diffusion of antibiotics from impregnated filter paper disks into the agar medium, with a bacterial growth inhibition zone observed on the agar surface. GST functions similarly to the DDM but uses an antibiotic-impregnated strip instead of commercial disks. The strip contains a predefined antibiotic concentration scale, which enables the determination of the bacterial isolate's sensitivity to the specific antibiotic. Although conventional methods are widely used for studying AMR, the tests primarily focus on phenotypic characteristics. To extend the genotypic characterization, molecular methods such as PCR and WGS have been recently incorporated into most AMR research studies (S. Fatima et al., 2023; Kumar et al., 2021; Lu et al., 2022; Z. Xu et al., 2020; Yu et al., 2024).

2. Aims of the thesis

This thesis aimed to enhance knowledge on the occurrence and AMR characteristics of *Salmonella* spp. isolated from fresh food products in Cambodia, thereby contributing to improved understanding of food safety risks in the region.

To achieve the main objective, the specific objectives were:

- 1) To determine the prevalence of *Salmonella* and its serovars distribution in raw meat, seafood/fish, and vegetables in Phnom Penh markets, and the occurrence of *Salmonella* at smallholder vegetable farms.
- 2) To investigate the cross-contamination risks related to *Salmonella* food contamination in markets and vegetable farms.
- 3) To identify the prevalence of *Salmonella* AMR and its ARGs in various fresh food commodities.
- 4) To characterize the identified *Salmonella* serovars' genetics, focusing on plasmid, virulence genes, and SPIs identification.

3. Materials and methods

3.1 Study design and frameworks

3.1.1 Study Area

Cambodia is situated in Southeast Asia, where it shares its borders with Laos, Vietnam, Thailand, and the Gulf of Thailand. The country experiences a tropical monsoon climate, with temperatures typically ranging from 21 to 35 °C (70 to 95 °F), reaching as high as 40 °C in April (NAP-GSP, 2022). Cambodia consists of 24 provinces and the capital, Phnom Penh, covering a total land area of 176,520km², of which 32% is cultivated land and 47% is covered by forest (Deryng et al., 2023). Phnom Penh encompasses an area of 678,46km² and has a total population of 1,307,713 individuals (CDC & Cambodia, 2013). Fresh food products are essential components of the Cambodian diet, which are readily available in local markets. There are three types of local markets: wholesale markets, retail markets, and superstores/supermarkets. Wholesale markets are open markets where fresh food products originating from different provinces and imported from neighboring countries are traded, while retail markets, in turn, obtain most of their food supplies from these wholesale markets (Sokhen et al., 2004). For superstores/supermarkets, most food products are well-packaged and stored under appropriate conditions, usually within enclosed facilities, reflecting the higher standards of these establishments compared to wholesale and retail markets. Most of the fresh food sold in these markets originates from different sources, including nearby livestock and vegetable farms around Phnom Penh, as well as from other provinces and neighboring countries such as Vietnam and Thailand.

The study was conducted in Phnom Penh City and the Kandal province, with fresh food samples collected from five local markets in Phnom Penh and two vegetable farms in the Kandal province that supply fresh produce directly to the markets (Figure 1). These two locations were selected based on their geographical proximity to the laboratory facility, which facilitated the handling and analysis of samples.

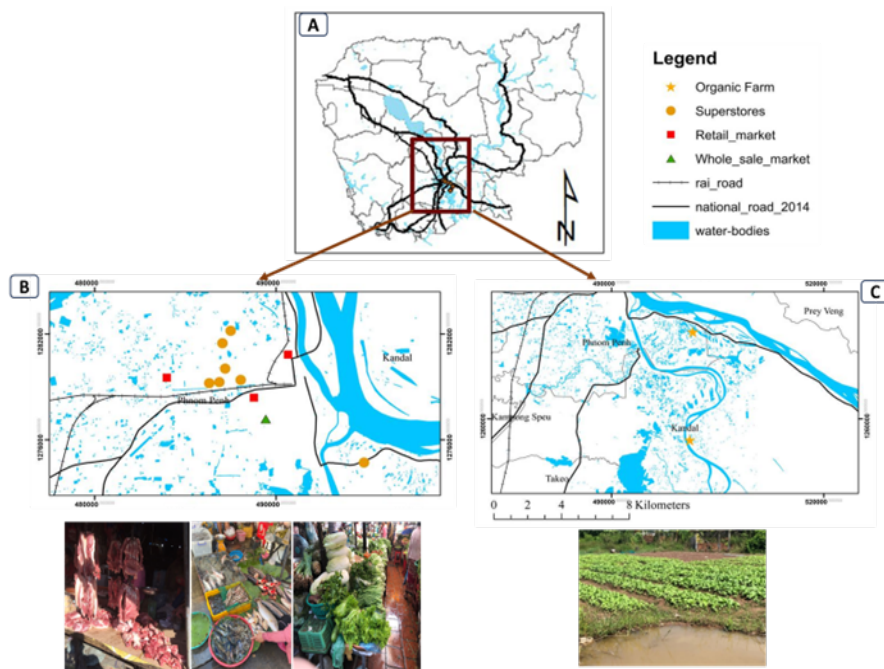


Figure 1. Map A is an overview of Cambodia, highlighting the sampling area. Map B shows the capital, Phnom Penh, and the markets that were sampled, while Map C displays the vegetable farms in Kandal province that were sampled, which support the local markets in Phnom Penh with leafy vegetables. (Map by Dr. Kongkea Phan, and pictures by RUPP-IU research team).

3.1.2 Study design

A cross-sectional study was conducted for fresh food sampling between November 2020 and November 2021. The present study aimed to collect at least 250 samples from different food categories across the selected markets and farms to estimate a prevalence of 80%, with a 95% confidence level and 5% precision. By evenly distributing samples across the five markets and two farms, a total of 285 samples were collected. Each market contributed 50 food samples, composed of 25 leafy vegetables, 15 meats, and 10 seafood/fish products, representing the major food types commonly consumed in Cambodia (Paper I). The study included vendors from various market types: 29 from the wholesale market, 69 from three retail markets (comprising 26, 24, and 19 vendors, respectively), and 11 from organic shops and supermarkets. The remaining 35 vegetable samples were collected from

two local farms in Kandal province to trace *Salmonella* contamination from farm to market.

All fresh food samples were processed within 24 hours of collection. Bacterial culture and confirmation tests were conducted at the microbiology laboratory in the Department of Food Chemistry, Faculty of Science and Technology, International University (IU), and the molecular genetics laboratory in the Department of Bioengineering, Faculty of Engineering, Royal University of Phnom Penh (RUPP). The molecular analyses and sequencing were performed at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden.

3.1.3 Study Workflow

This project commenced by assessing the overall prevalence of *Salmonella* in meat, seafood/fish, and vegetables obtained from local markets and farms (Paper I). Following this, all confirmed *Salmonella* isolates were examined for AMR phenotypes, and those possessing high-quality DNA that exhibited resistance to at least one antibiotic were prioritized for serotype identification and prediction of ARGs (Paper II). In the subsequent study, the identified *Salmonella* serovars underwent further genetic characterization, focusing on the detection of plasmids, virulence genes, and SPIs (Paper III). The study workflow from studies I to III is presented in Figure 2.

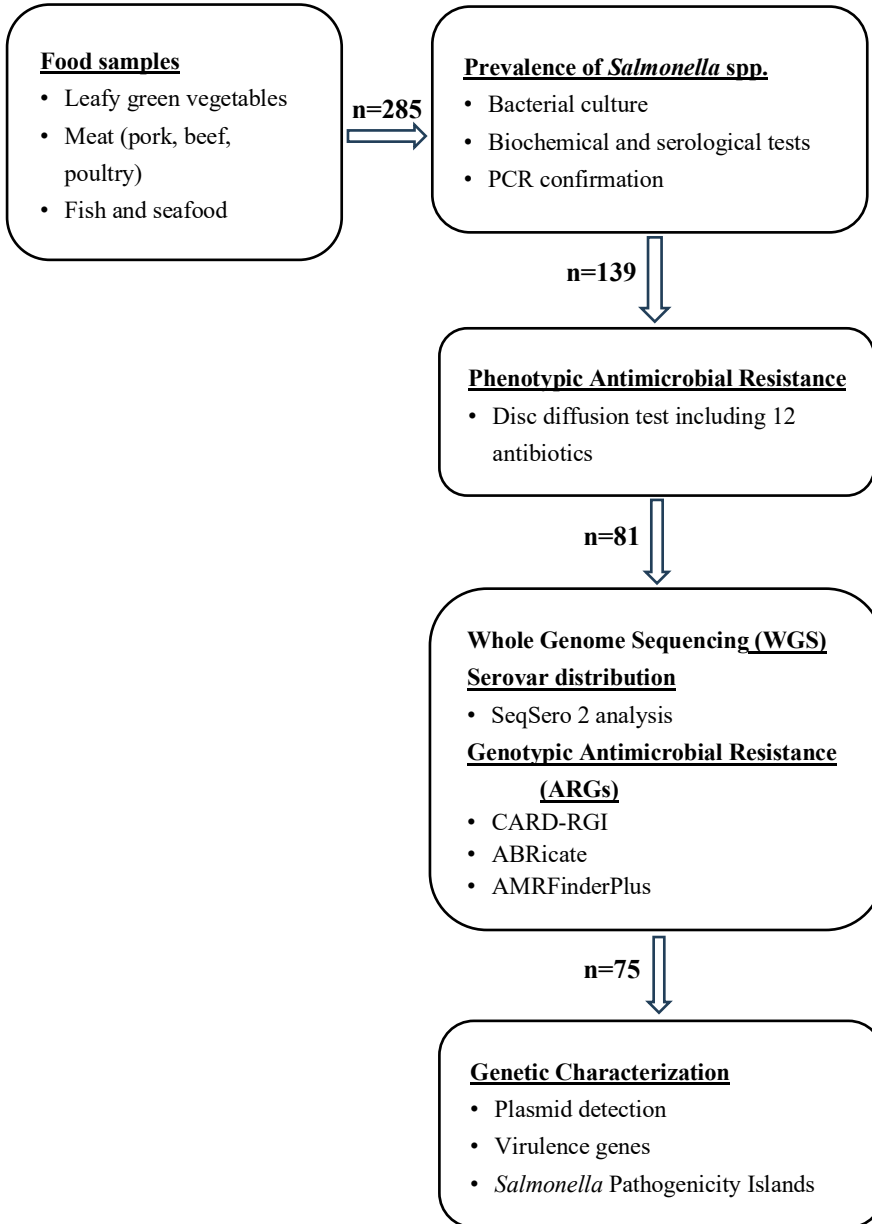


Figure 2. Workflow of the research study (Papers I-III). The number of samples and isolates was present in each study part. For example, 81 *Salmonella* isolates were used for WGS analysis to detect serovars and ARGs.

3.2 *Salmonella* prevalence and serovar distribution (Papers I & II)

3.2.1 Prevalence of *Salmonella* spp. among different food commodities

In Paper I, a total of 285 food samples (meat, seafood/fish, and vegetables) were collected from five local markets in Phnom Penh and two farms in Kandal province. Of these samples, 125 were either meat (n = 75) or seafood/fish (n = 50), which were obtained from local markets. Additionally, 160 vegetable samples were obtained from both local markets and farms. Each sample, weighing approximately 500g, was immediately placed in a clean plastic bag and stored in a cool box with ice packs for a maximum of 5 hours prior to being transported to the laboratory for processing and analysis.

Salmonella isolation

Salmonella was cultured according to the ISO 6579-1:2017 method. In summary, each sample was cut into small pieces and pre-enriched in buffer peptone water (BPW, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Aliquots were then transferred into selective enrichment broths and incubated for 18-24 hours in MKTTn (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) at 37°C and in RVS (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) at 42 °C. Subsequently, each culture was streaked onto selective agar plates, including XLD (HiMedia, Maharashtra, India) and BGA (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), for identification of typical *Salmonella* colonies. Each typical colony was sub-cultured to acquire pure *Salmonella* stock cultures for subsequent identification analyses.

Salmonella confirmation

Presumptive *Salmonella* colonies from each pure culture were confirmed using microscopic morphology identification, latex slide agglutination commercial LK02-HiSalmonella™ Latex test kit (HiMedia, Maharashtra, India), and biochemical test with KBM002 Himotility™ Biochemical kits for *Salmonella* (HiMedia, Maharashtra, India). The *Salmonella* isolates were confirmed using PCR with *invA* primers (Paper I).

3.2.2 *Salmonella* serotype distribution

Once the isolates were confirmed, Latex agglutination was performed with reagents from the *Salmonella* Sero-Quick Group kit (SSI, Diagnostica A/S, Hillerød, Denmark) to identify the most common *Salmonella* serogroups (Paper I). A total of 81 *Salmonella* isolates containing high-quality DNA were selected as priority strains demonstrating resistance to at least one antibiotic and a non-resistant reference strain. These isolates were then subjected to WGS to identify their serotypes (Illumina, SciLifeLab, Uppsala, Sweden). *Salmonella* serotypes were predicted using SeqSero2 with its reference database (Paper II).

3.3 *Salmonella* cross-contamination (Paper I)

To investigate potential cross-contamination associated with the occurrence of *Salmonella* in the examined food products, observations and short interviews were carried out during the sampling period (Paper I). Pre-made checklists were used to document relevant factors, including actual temperature at the sampling sites, types of fresh food containers, and potential vectors for *Salmonella* transmission. Furthermore, the short interview addressed the origin of the food products, the time between harvest and sale to vendors, and the frequency of hygiene and sanitation practices at each sampling location.

3.4 Antimicrobial Resistance (Papers II & III)

3.4.1 AMR phenotype

A total of 139 *Salmonella* isolates were tested for AMR profile using antimicrobial susceptibility tests (ASTs). The disk diffusion method (Kirby-Bauer) was performed with commercial Whatman filter paper disks impregnated with defined antibiotic concentrations. Twelve antibiotics were included in this study (Table 1), representing seven antibiotic classes commonly used to treat *Salmonella* infections (Punchihewage-Don et al., 2024). The antibiotics frequently used to treat human *Salmonella* are azithromycin, ciprofloxacin, aztreonam, cefuroxime, amoxicillin, and ampicillin (Sivanandy et al., 2025; Yoon et al., 2009) (Paper II). The inhibition zone was measured and interpreted according to the Clinical

Laboratory Standards Institute (CLSI) (Table 2). Resistance to three or more antibiotics was classified as MDR.

Table 1. The CLSI standard inhibition zone for selected antibiotics used in this study.

Antimicrobial Class	Antimicrobial		Disk Content (ug)	Zone Diameter Breakpoints, nearest whole mm		
	Sub-Class	Antibiotic Agent	Abbrevia- tion	Susceptible (S)	Intermediate (I)	Resistance- (R)
Beta-lactam	Monobactams ^a	Aztreonam	Atm	≥21	18-20 ^b	≤17
	Cephem ^a (Cephalosporin)	Cefuroxime	Cxm	≥18	15-17 ^b	≤14
	Penicillin ^a	Ampicillin	Amp	≥17	14-16	≤13
		Amoxicillin	Aml	≥18	14-17 ^b	≤13
	Carbapenems ^a	Imipenem	Ipm	≥23	20-22 ^b	≤19
Folate Pathway Antagonists		Sulphamethoxazole-Trimethoprim	Sxt	≥16	11-15	≤10
Macrolides		Azithromycin	Azm	≥13		≤12
Quinolones		Ciprofloxacin	Cip	≥31	21-30 ^b	≤20
Phenicol		Chloramphenicol	C	≥18	13-17	≤12
Tetracycline		Oxytetracycline	Ot	>15	12-14	<11
		Doxycycline	Do	≥14	11-13	≤10
Aminoglycosides		Gentamycin	Gn	≥15	13-14 ^b	≤12

^a monobactam, carbapenems, cephalosporin, and penicillin are subclasses of beta-lactam antibiotics.

^b Intermediate break points for corresponding antibiotic substances that can potentially concentrate at an anatomical site.

^c the concentration of mixed antibiotics with the proportion 1:19 of Trimethoprim/Sulfamethoxazole.

3.4.2 AMR genotype

A total of 81 out of 139 *Salmonella* isolates were subjected to ARGs identification (Paper II) (see Section 3.2.2 for the rationale behind the selection of these isolates). In brief, DNA was extracted from the selected isolates using the commercial Wizard® HMW DNA extraction kit (Promega, Madison, USA). Samples that met the required quality and quantity standards were sequenced on the Illumina NovaSeq X platform using a 10B lane with 150-cycle paired-end sequencing (Illumina, SciLife Lab, Uppsala, Sweden). Raw Illumina sequencing reads were quality-checked using FastQC (Andrews, 2010) and trimmed with Trimmomatic (Bolger et al., 2014), which removed adapter sequences and filtered out low-quality reads (Phred score < 25) using default parameters. After this, Genome assemblies were generated using SPAdes v3.15.5. Assemblies with N50 > 30kb and fewer than 500 contigs were considered for downstream ARG prediction. Three ARG prediction tools, namely Comprehensive Antibiotic Resistance Database (CARD) Resistance Gene Identifier (RGI) (Alcock et al., 2020), AMRFinderPlus, and ABRicate v1.0.1, were used in this study to ensure comprehensive detection and to reduce the risk of missing gene predictions (Papers II and III) (Feldgarden et al., 2021; Liu et al., 2019).

3.5 Genetic characterization (Paper III)

A total of 75 *Salmonella* isolates were included in the genetic characterization, as they provided a clear serovar distribution with high AMR phenotypic and genotypic characteristics in studies I and II. Thus, to investigate the genetic context of resistance, these 75 identified *Salmonella* serotypes were further characterized using PlasmidFinder, which was used to identify plasmid replicons (Carattoli & Hasman, 2020); VFAnalyzer/Virulence Finder v2.0.4 screened for virulence genes using curated virulence factor references (Joensen et al., 2014), and ABRicate v1.9.1 with VFDB for virulence genes (Liu et al., 2019; Seemann, 2018); SPIFinder detected SPIs using a dedicated SPI reference database (Roer et al., 2016).

All sequence analyses described in the Materials and Methods section 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.

4. Results and discussion

4.1 *Salmonella* prevalence and serovar distribution (Papers I & II)

4.1.1 Prevalence of *Salmonella* spp. among different food commodities

Of the 285 samples, 138 (48.4%) were found to be positive for *Salmonella* (Table 2). The sequential test results demonstrated a reduced number of positive samples at each stage: 255/285 were initially positive by culture-based methods, 174/255 were confirmed through biochemical/serological testing, 173/174 underwent AMR testing, and a final confirmation was obtained by PCR. A similar study from India showed a decline in *Salmonella* detection rates when the traditional culture method was compared against molecular approaches (Tiwari et al., 2022). In that study, the apparent prevalence on broiler farms decreased from 20% by culture-based testing to 13.3% by molecular methods, while in layer farms, detection dropped from 45.4% to 36.3%. These findings demonstrate that culture-based methods alone cannot accurately confirm the presence of *Salmonella* contamination. Subsequent confirmatory tests are necessary to ensure reliable results, emphasizing the importance of standardized laboratory procedures and well-equipped facilities for accurately detecting *Salmonella*, even at low concentrations, in various sample sources.

Table 2. The sequential test result of each sample type and the corresponding prevalence of *Salmonella* spp.

Sample Types	No. Samples	Culture-positive samples	Total sample (%) Positive for each test		
			Biochemical/Serological	Antibiotic Resistance	PCR Confirm
Meat	75	72 (96%)	53 (70.7%)	53 (70.7%)	53 (70.7%)
Seafood/fish	50	50 (100%)	32 (64%)	32 (64%)	32 (64%)
Vegetable Markets	125	108 (86.4%)	46 (36.8%)	45 (36%)	42 (33.6%)
Vegetable Farms	35	25 (71.4%)	11 (31.4%)	11 (31.4%)	11 (31.4%)
Total	285	255 (89.5%)	174 (61%)	173 (60.7%)	138 (48.4%)

The biochemical characteristics of all confirmed *Salmonella* isolates by PCR test showed that all isolates tested positive for both catalase and citrate utilization (Table 3). Between 90-99% of the isolates were positive for carbohydrate utilization (trehalose, arabinose, and maltose), lysine utilization, and hydrogen sulfide (H₂S) production strains. Most of the isolates in this study were motile, with only 9% (13/139) identified as non-motile. This finding is similar to the study on *Salmonella* spp. Isolated from chevon, mutton, and environments from the retail market in India, where all isolates were positive for both the catalase and citrate tests (Makwana et al., 2015). Another study also showed that *Salmonella* isolated from broiler chicks in Egypt were positive for citrate, catalase, lysine Iron agar, maltose, L-arabinose, but negative for urease and beta-galactosidase (ONPG) (Sedeik et al., 2019).

Table 3. Biochemical characteristics of *Salmonella enterica* (n = 139)

No.	Name of test	Typical <i>Salmonella enterica</i> reactions*	No. (%) of isolates
1	Catalase	+	139 (100 %)
2	Motility	+	126 (91%)
3	Citrate utilization	+	139 (100 %)
4	Urease	-	111 (80%)
5	Arginine	v ^a	54 (39%)
6	Lysine	+	134 (96%)
7	H ₂ S Prod.	+	137 (99%)
8	ONPG	-	102 (73%)
9	Arabinose	+	132 (95%)
10	Lactose	-	137 (99%)
11	Maltose	+	126 (91%)
12	Trehalose	+	137 (99%)

* Reference sources: (Mikoleit, 2014; Sedeik et al., 2019)

^a mean 11-89% variable result

In terms of prevalence, *Salmonella* contamination was confirmed in 53/75 (71%) of meat samples, 32/50 (64%) of seafood/fish samples, and 53/160 (33%) of vegetable samples (Table 2). The notably high contamination rate in meat samples suggests that it may serve as a primary source of foodborne infections. This observation is consistent with previous studies, including one that reported a 47% prevalence of *Salmonella* among pigs, broiler chickens, and meat products in local fresh markets in the Thailand-Cambodia

border provinces (Trongjit et al., 2017). Similarly, a study across 52 traditional markets in 25 Cambodian provinces found a 42% prevalence in chicken and pork samples (224/532) (Chea et al., 2021). A meta-analysis of 49 studies in China also reported high *Salmonella* contamination in raw meat products (23.6%), compared to other food commodities (Miao et al., 2022). The high prevalence of *Salmonella* contamination in fresh food products may be due to contamination occurring from pre-harvest to post-harvest stages, as well as improper food handling at the market level.

4.1.2 *Salmonella* serovar distribution

Among the 81 isolates that were further typed, *Salmonella* serogroups were identified using two approaches. Latex agglutination detected six serogroups (A, B, C, D, E, and G) plus UNC, while WGS analysis identified seven serogroups (B, C, E, F, G, I, and R) plus UNC (Figure 3). Both methods indicated that serogroups B (33% and 21%) and C (35% and 44%) were the most prevalent. Serogroups A and D were identified solely through latex agglutination, whereas serogroups F, I, and R were detected exclusively by WGS analysis. Inconsistent serogroup identification was also observed. For example, while WGS classified nine isolates as serogroup E, the latex agglutination method identified six of these as serogroup B, two as serogroup C, and one as serogroup D. A comparative study between WGS and traditional serotyping showed that WGS analysis using SeqSero2 achieved a serotype prediction accuracy of 98%. In line with our findings, traditional serotyping remains challenging, as it requires specific antisera and highly trained laboratory personnel (Diep et al., 2019). Other studies have also demonstrated the high accuracy of WGS tools such as SeqSero2 and SISRT, highlighting their potential for reliable *Salmonella* serotype identification (Ibrahim & Morin, 2018; Kagambèga et al., 2021; Lamas et al., 2023). The discrepancies in detection and prevalence between the two approaches underline the limitations of the conventional latex agglutination method compared with the advanced molecular approach of WGS.

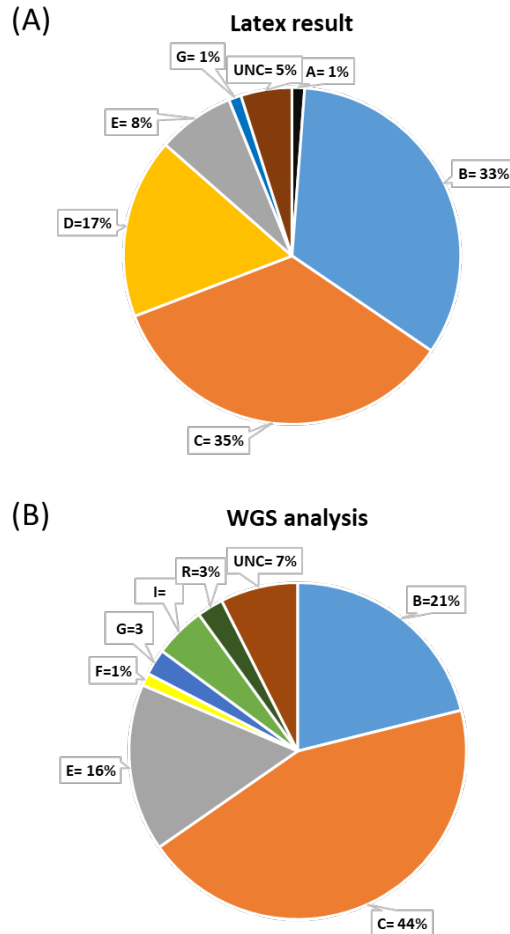


Figure 3. Percentage of Serogrouping found among 81 *Salmonella* spp. isolates: (A) result from the latex agglutination test, and (B) result from WGS analysis. UNC represents non-classified serogroups.

Moreover, WGS analysis identified 32 serotypes among 75 of the 81 *Salmonella* isolates, while six isolates could not be classified (Figure 4). The six most common serotypes were: *S. Corvallis* (meat and vegetables), *S. Weltevreden* (seafood/fish and vegetables), *S. Haifa* (meat, seafood/fish, and vegetables), *S. Agona* (meat), *S. Livingstone* (meat and seafood/fish), and *S. Kentucky* (meat, seafood/fish, and vegetables) (Figure 4). Previous studies in Cambodia have underscored the identification of various *Salmonella* serovars across different food sources. Indeed, Lay et al. (2011) found five

dominant serovars in poultry carcasses: *S. Anatum*, *S. Typhimurium*, *S. Corvallis*, *S. Stanley*, and *S. Enteritidis*, while Lay et al. (2021) reported *S. Rissen* and *S. Anatum* as the most common in pigs and pork from the local market along border areas. Similarly, another study on broiler chickens, pigs, and meat products in Thailand-Cambodia border provinces reported that the two most common serovars were *S. Typhimurium* and *S. Rissen* (Trongjit et al., 2017). Another report found that the five most common serovars isolated from humans, animals, and food were *S. Typhi*, *S. Derby*, *S. Schwarzengrund*, *S. Stanley*, and *S. Weltevreden*, indicating possible cross-transmission among these sources (van Cuyck et al., 2011). Additionally, a study on food contact and non-contact surface objects, such as concrete walls, metal or wood fencing sustaining the roof, and open-air tents, in local markets identified six most common serovars: *S. Rissen*, *S. Hvittingfoss*, *S. Corvallis*, *S. Krefeld*, *S. Weltevreden*, and *S. Altona* (Schwan et al., 2021).

Beyond Cambodia, a study conducted in Singapore reported distinct *Salmonella* serovars associated with specific food commodities: *S. Enteritidis* in chicken and chicken products, *S. Typhimurium* and *S. Derby* in pork and pork products, and *S. Weltevreden* in seafood. These findings suggest that certain serovars are often linked to specific food types (Aung et al., 2020). Studies performed in Vietnam documented the common serovars across human, animal, and environment samples, including *S. Weltevreden*, *S. Typhimurium*, *S. Derby*, *S. London*, *S. Anatum*, *S. Rissen*, *S. Enteritidis*, *S. Albany*, and monophasic *S. Typhimurium* (Nguyen et al., 2021; Nhung et al., 2024). In addition, studies from southern Italy reported the presence of *S. Infantis*, *S. Derby*, *S. Typhimurium*, monophasic *S. Typhimurium*, and *S. Rissen* in carcass-based food products and other foods, further highlighting the global diversity of circulating serovars (Peruzy et al., 2022). The present findings indicate that *S. Weltevreden* was also identified in vegetables from local markets as well as at the farm level, suggesting that contamination may occur early in the production chain. The fact that this serovar has been reported in various samples, including meat, humans, animals, and the environment, in countries aside from Cambodia (Ferrari et al., 2019; Jain et al., 2015; Thong et al., 2002; Zhang et al., 2023), illustrates its potential significance as a public health concern.

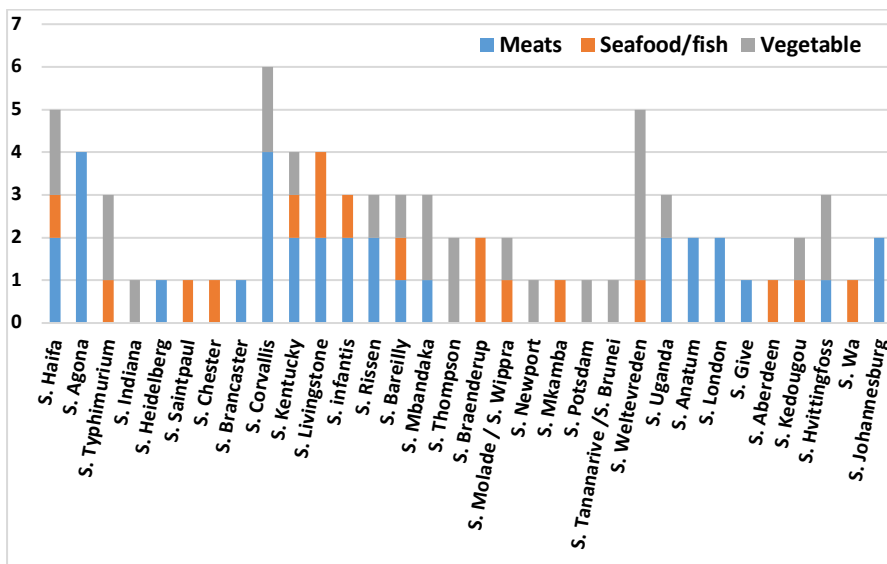


Figure 4. Serovars distribution among *Salmonella* isolates from different food commodities in this study.

4.2 Potential risk for *Salmonella* cross-contamination (Paper I)

Based on the checklist and the brief interviews conducted during the food sampling, potential risks of cross-contamination with *Salmonella* have been examined (Figure 5). One key issue is the lack of adequate cool storage in most local markets, as only 6% of the study vendors were noted to contain cold storage. This not only promotes microbial growth but also accelerates the spoilage of fresh food products. Together with microbial risks, several market handling practices may facilitate cross-contamination. Various transportation methods were also observed, primarily under open-air conditions, which included motorcycles, trailers, tuk-tuks, and bicycles for short distances, while cars were used for longer distances. Only about 27% of the vendors reported using full personal protective equipment (PPE), such as masks, gloves, and aprons, during food handling, which may reduce their risk of contamination at this process stage. Vegetables washed or rinsed with contaminated water represent a major source of foodborne pathogen transmission (Osafo et al., 2022). In this study, 49% of vendors rinsed vegetables with tap water, while less than 3% used pond water to remove dirt

and maintain freshness. The remaining vegetables were kept in their original farm-supplied containers. Mixing different types of food in the same container was also seen, a practice that can further promote cross-contamination and the spread of foodborne pathogens. Previous studies have reported that microbial contamination in fresh vegetables is influenced by multiple factors, including the application of manure (containing bacteria that can contaminate food) as fertilizer, inadequate storage conditions, insufficient hygiene and sanitation practices, low-quality market infrastructure, and improper techniques for cutting vegetables (Alegbeleye et al., 2018; Desiree et al., 2020). A microbial risk assessment further suggested that cross-contamination may occur during food preparation or from unwashed or improperly cleaned raw materials (Chea et al., 2022). These results underscore the need for targeted intervention strategies to strengthen food safety regulations.

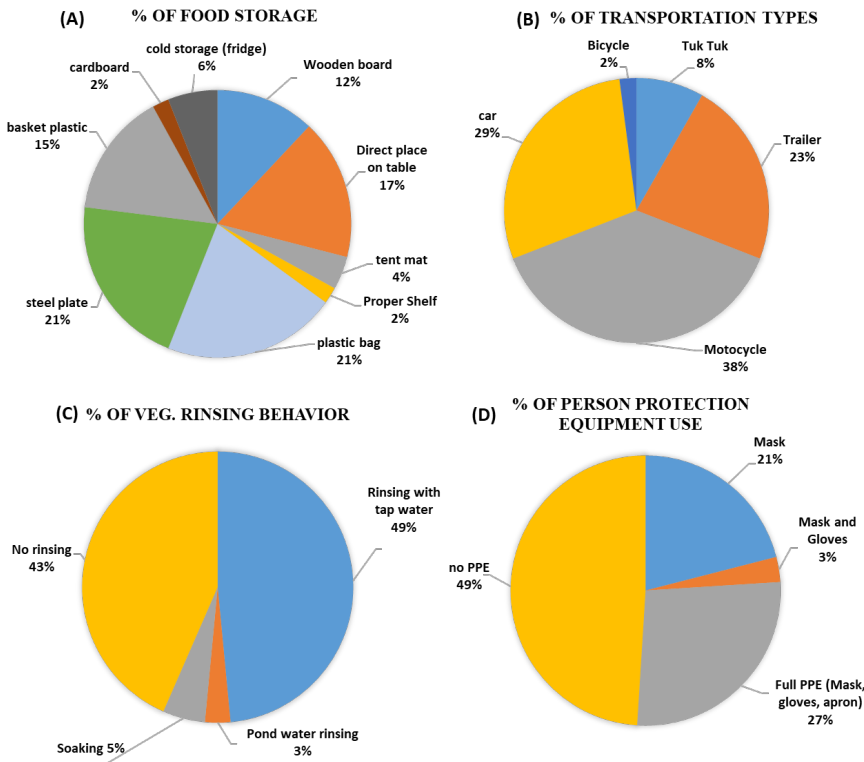


Figure 5. Potential risk factors for *Salmonella* cross-contamination, as identified in the current study.

4.3 *Salmonella* antimicrobial resistance (Paper II & III)

4.3.1 AMR phenotypes

The increasing prevalence of AMR *Salmonella* in LMICs constitutes a serious public health threat, complicates the treatment of infections, and places additional burdens on healthcare systems and disease management efforts. In the present study, AMR profiling was conducted on 139 *Salmonella* isolates. The results revealed a high prevalence of resistance, with 71% (99/139) of isolates resistant to at least one antibiotic, and 39% (39/99) classified as MDR.

The highest prevalence of resistance was reported for macrolides (azithromycin) at 37%, followed by tetracyclines at 35%, and β -lactams (ampicillin and amoxycillin) at 24%. Similarly, a study in Cambodia found that *Salmonella* isolates from swine and poultry farms exhibited high AMR prevalence, with resistance rates ranging from 73.8% to 100% for β -lactams, tetracyclines, and sulfonamides (Chea et al., 2025). Isolates from vegetable sources demonstrated a notably high resistance to azithromycin compared with those from seafood/fish and meat. In contrast, a higher proportion of isolates from meat showed resistance to tetracycline than those from vegetables and seafood/fish. A high prevalence of AMR *Salmonella* spp. has been documented worldwide. In Vietnam, the prevalence of AMR among non-typhoidal *Salmonella* isolates increased between 2000 and 2020, with a reported resistance rate of 15.6% to quinolones, 23.7% to cephalosporins, 16.1% to penicillins, 12.9% to tetracyclines, and 11.4% classified as MDR (Nhung et al., 2024). Likewise, studies in South Asia identified high AMR levels, with 70% of *Salmonella* isolates from human, animal, and environment samples demonstrating resistance, including 74.3% to nalidixic acid and 37.6% to tetracycline (Talukder et al., 2023). In Europe, *Salmonella* isolates collected between 1900 and 2023 showed a high prevalence of AMR, with 52.9% resistant to fluoroquinolone, followed by 21.3% to tetracycline, 19.5% to sulfonamide, and 18.7% to β -lactam (Kumar et al., 2025; Wang et al., 2025). Overall, these findings clearly reflect the urgent need for targeted interventions and strengthened food safety and antimicrobial stewardship measures to limit the spread of resistant *Salmonella* strains.

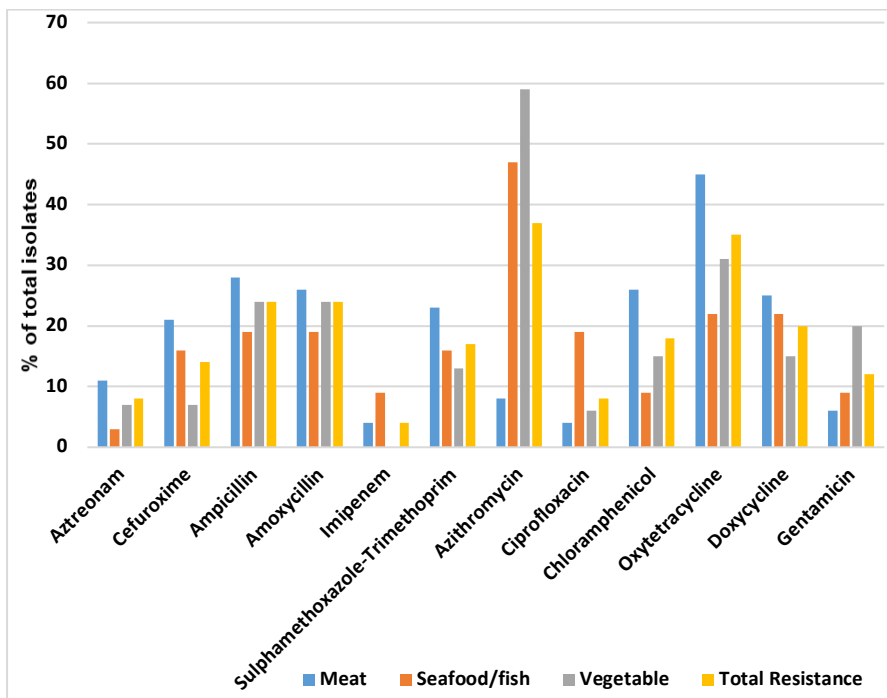


Figure 6. Antimicrobial resistance (AMR) percentage detected in the 139 *Salmonella* spp. isolates from different meats, seafood/fish, and vegetables in Cambodia.

4.3.2 AMR genotypes

Based on the AMR findings, 81 *Salmonella* isolates were subjected to WGS, and three analysis tools (CARD-RGI, AMRFinderPlus, and ABRicate) were used for ARG identification. Of these tools, CARD-RGI was the most efficient, detecting a higher number of ARGs than the other two. For example, CARD-RGI successfully identified all genes linked to resistance against macrolides, beta-lactams, quinolones, and aminoglycosides in *Salmonella* strains. Regarding tetracycline resistance, CARD-RGI identified all 40 isolates, whereas ABRicate identified 31, and AMRFinderPlus identified 29.

Figure 7 presents the most common ARGs predicted through CARD-RGI, with the other tools supplementing the few genes not captured by CARD-RGI. Most *Salmonella* isolates carried MDR genes, including *sdiA*, *rsmA*, *acrB*, *marA*, *emrA*, *emrB*, *emrR*, *MdtQ*, *acrD*, *baeR*, *cpxA*, *kdpE*, *gols*,

mdsA, *mdsB*, and *mdsC*. This study revealed the presence of numerous specific resistance genes that may contribute to the mechanisms underlying *Salmonella*'s antibiotic resistance. Common ARGs linked to macrolide (azithromycin) resistance included *CRP*, *mphA*, *msrC*, *Mrx*, and *ErmB*. Tetracycline resistance was associated with *tet* genes (A, B, M, 45, and X4) and *adeF*. Resistance to β -lactam was conferred by genes such as *TEM*-(1, 176, and 215), *CTX-M* (55 and 65), *CMY-159*, *Sed-1*, *SHV-26*, *LAP-2*, and *OXA* (1 and 10). For the folate pathway antagonist, the identified ARGs were *dfrA* (1, 12, and 14) and *sul* (1,2, and 3). The common ARGs for phenicols included *floR*, *cmlA*, and *catB3*, while quinolone/fluoroquinolone resistance genes were *qnrS* (1 and 2), *qnrD1*, *qnrB19*, and *qepA2*. Lastly, aminoglycoside resistance genes were found to be *AAC*, *APH*, and *aad* (1,2,3,7,16, and 23).

Consistent with the findings mentioned above, studies on *Salmonella* from various common sources, such as human, avian, environmental, water, swine, and food, have detected several prevalent ARGs across different antibiotic classes. These include β -lactam (*blaTEM-1B*), fluoroquinolone (*parC* and *qnrB19*), folate pathway antagonist (*sul2*), macrolide (*mphA*), phenicol (*floR*), polymyxin B (*mcr-1.1*), and tetracycline (*tet(A)*) (Mąka & Popowska, 2016; Nuanmuang et al., 2023). Further, a study on ARGs among *Salmonella* isolated from four food animals in China showed that the 4 most common ARGs were *tetB*, *sul2*, *aadA2*, and *aph(3')-IIa*, while the remaining ARGs included β -lactam (*blaTEM*, *blaSHV*, *blaCMY-2*, *blaCTX-M*, and *blaOXA*), and quinolone resistance genes (*oqxA*, *oqxB*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *qepA*) (Guo et al., 2023).

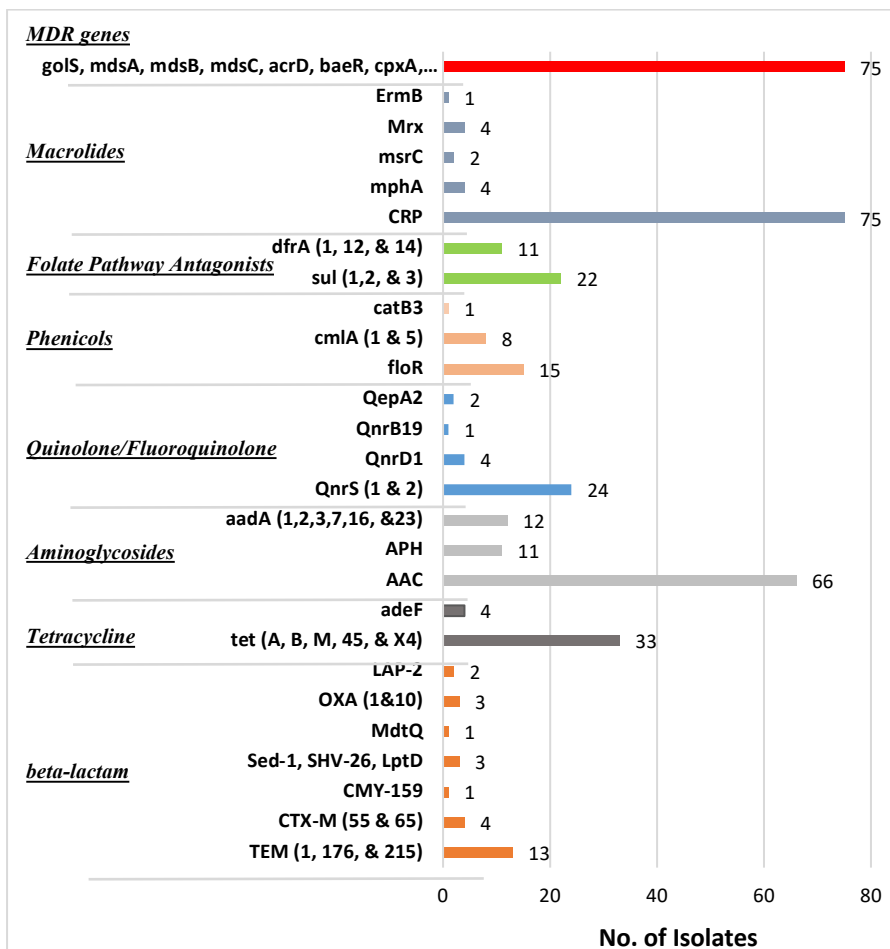


Figure 7. Common ARGs were identified among 32 *Salmonella* serovars (n = 81 isolates) isolated from different food commodities used in this study. Different-colored bars indicate different genes in various antibiotic classes.

To summarize, WGS analysis revealed ARGs that are responsible for six main resistance mechanisms. Of these, antibiotic efflux and target alteration were present in 99% of isolates, antibiotic inactivation in 96%, target protection 48%, target replacement 31%, and reduced permeability in only 2% (Table 4). This suggests that efflux pumps, target alteration, and antibiotic inactivation are the primary mechanisms through which *Salmonella* progresses from resistance to a single antibiotic to MDR. Efflux pumps act as the bacterium's first line of defense, preventing many antibiotics from entering the cell. Therefore, understanding *Salmonella*

efflux pumps will in turn provide insights into its virulence, biofilm formation, and eventual development into MDR strains (Alenazy, 2022; Chaudhari et al., 2023). Recently, nine types of efflux pumps have been identified, namely *AcrAB*, *AcrEF*, *AcrD*, *MdsABC*, *MdtABC*, *EmrAB*, *MdfA*, *MacAB*, and *MdtK*, each of which contributes to resistance against different classes of antibiotics and other virulence mechanisms (Zhou et al., 2023). Among these, *AcrAB* is primarily associated with resistance to fluoroquinolones and β -lactams, whereas *MacAB* is associated with macrolides and antioxidant stress.

Table 4. Antibiotic resistance mechanism and common ARGs found among 81 *Salmonella* spp. Isolates

Mechanisms	No. of isolates	% of isolates	Gene family
antibiotic inactivation	78	96%	<i>AAC(3)-Iie</i> , <i>AAC(3)-IId</i> , <i>AAC(3)-Iva</i> , <i>AAC(6')-Iy</i> , <i>AAC(6')-Iaa</i> , <i>AAC(6')-Ii</i> , <i>AAC(6')-Ib10</i> , <i>AAC(6')-Iid</i> , <i>AAC(6')-If</i> , <i>aadA</i> , <i>aadA(2,3,7,23)</i> , <i>APH(3')-Ia</i> , <i>APH(3')-Ib</i> , <i>APH(4)-Ia</i> , <i>APH(6)-Id</i> <i>CMH-3</i> , <i>CTX-M-55</i> , <i>CTX-M-65</i> , <i>LAP-2</i> , <i>OXA-1</i> , <i>OXA-10</i> , <i>SHV-11</i> , <i>SHV-26</i> , <i>Sed-1</i> , <i>CMY-159</i> , <i>TEM-1</i> , <i>TEM-176</i> , <i>TEM-215</i> , <i>ACC-1a</i> <i>tet(X4)</i> , <i>mphA</i> , <i>Mrx</i> , <i>lnuA</i> , <i>catA4</i> , <i>catB3</i> , <i>catII</i> from <i>E. coli</i> K-12, <i>qrr-2/-3</i> , <i>E.coli ampC</i> beta-lactamase
antibiotic efflux	80	99%	<i>kdpE</i> , <i>mdtABC</i> , <i>acrABDEFS</i> , <i>cpxA</i> , <i>baeRS</i> , <i>LptD</i> , <i>qacEdelta1</i> , <i>qacGL</i> , <i>emrABR</i> , <i>QepA2</i> , <i>MdtK</i> , <i>sdiA</i> , <i>rsmA</i> , <i>adeF</i> , <i>oqxAB</i> , <i>E. coli acrA</i> , <i>Shigella flexneri acrA</i> , <i>tet(A)</i> , <i>tet(B)</i> , <i>tet(J)</i> , <i>tet(L)</i> , <i>tet(45)</i> , <i>emrK</i> , <i>E. coli (mdfA, emrE)</i> , <i>efrA</i> , <i>efmA</i> , <i>mef(B)</i> , <i>H-NS</i> , <i>CRP</i> , <i>mdsABC</i> , <i>golS</i> , <i>flrR</i> , <i>cmlA1</i> , <i>cmlA5</i> , <i>msbA</i> , <i>leuO</i> , <i>mdtMPG</i> , <i>evgA</i> , <i>gadW</i> , <i>KpnEFGH</i>
antibiotic target alteration	80	99%	<i>ErmB</i> , <i>MCR-1.1</i> , <i>ArnT</i> , <i>PmrF</i> , <i>ugd</i> , <i>eptB</i> , <i>bacA</i> , <i>vanG</i> , <i>Haemophilus influenzae</i> PBP3 conferring resistance to beta-lactam antibiotics, <i>E. coli</i> EF-Tu mutants conferring resistance to Pulvomycin, <i>S. isangi gyrA</i> , <i>S. enterica gyrA</i> , <i>E. coli gyrA</i> , <i>E. coli parC</i> , <i>Morganella morganii gyrB</i> , <i>vanY</i> gene in (<i>vanA/vanB/vanM/vanF</i>) cluster, <i>vanXY</i> gene in <i>vanC</i> cluster, <i>vanT</i> gene in <i>vanG</i> cluster
antibiotic target replacement	25	31%	<i>dfrA1</i> , <i>dfrA12</i> , <i>dfrA14</i> , <i>dfrE</i> <i>sul1</i> , <i>sul2</i> , <i>sul3</i>
antibiotic target protection	39	48%	<i>QnrS1</i> , <i>QnrS2</i> , <i>QnrD1</i> , <i>QnrB12</i> , <i>QnrB19</i> <i>tet(M)</i> , <i>lsaA</i> , <i>msrC</i> , <i>eatAv</i>
reduced permeability to antibiotic	2	2%	<i>MdtQ</i> , <i>OmpA</i> , <i>Klebsiella pneumoniae OmpK37</i>

4.4 *Salmonella* genetic characterization (Paper III)

4.4.1 Plasmids identified among *Salmonella* serovars

The present study identified 22 plasmids across 32 *Salmonella* serovars, with Col(pHAD28) being the most prevalent, which was detected in 23 serovars, followed by Col440I in 10 serovars and IncFII(S) in 6 serovars (Figure 8). The mobilizable plasmid Col(pHAD28) has been previously reported in different *Salmonella* strains (Habib et al., 2024; Jibril et al., 2024; Liu et al., 2024). The Col440I plasmid has been detected in *Salmonella* isolated from pork and chicken, carrying plasmid-mediated quinolone resistance (PMQR) that can reduce susceptibility to quinolones in *Salmonella* (Chung et al., 2024; Lyu et al., 2021). 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, out of the 75 *Salmonella* isolates analyzed in this study, 13 showed no detectable known plasmids. These included 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*. Four *Salmonella* isolates, two *S. Typhimurium*, one *S. Weltevreden*, and one *S. Mbandaka*, were found to carry the IncFIB(S) plasmid. This finding is in line with previous studies reporting the presence of the IncFIB plasmid in *S. Typhimurium*. This virulence-associated incompatibility plasmid has been linked to the slower development of AMR in specific serovars (Chung et al., 2024; Rychlik et al., 2006). Taken together, these findings suggest that Col and Inc plasmids may be linked to the dissemination of AMR among *Salmonella* strains.

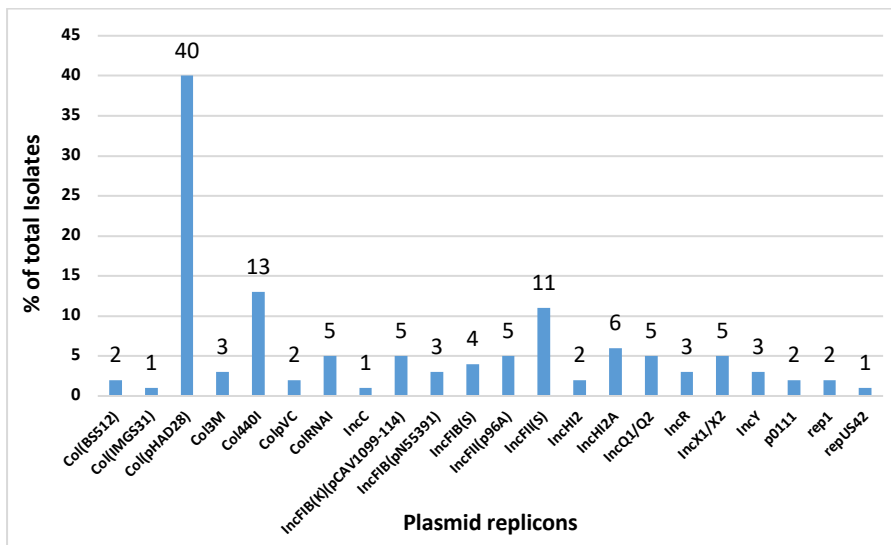


Figure 8. Plasmid replicons were identified among 32 *Salmonella* serovars (n = 75 isolates) isolated from different food commodities used in this study.

4.4.2 Virulence genes identification

A total of 150 virulence genes were detected across *Salmonella* serovars, and these were categorized into eight functional groups, with the remaining unclassified genes placed under “others” (Table 5). Most virulence genes were associated with the TTSS, fimbrial and non-fimbrial adherence, macrophage-inducible genes, transporter genes, and magnesium uptake. Most TTSS genes were present across all *Salmonella* serovars, except *avrA*, which lacked *S. Livingstone*, *S. Indiana*, *S. Newport*, and *S. Mbandaka*. Along with TTSS, several virulence genes were consistently found in all serovars, such as fimbrial adherence genes (*fimCDFHI*), non-fimbrial adherence genes (*sinH*, *misL*), the macrophage-inducible gene (*mig-14*), transporter genes (*csgABCDEFHG*, *fepCG*), and magnesium uptake genes (*mgfBC*). Similarly, a broad range of virulence genes with specific functions was identified among *Salmonella* isolates from both animal and humans in China, as well as the two *Salmonella* serovars (*S. Gallinarum* and *S. Pullorum*) isolated from poultry sources (Campos et al., 2024; Yan et al., 2022). Some genes, however, were restricted to specific serovars. For instance, the *cdtB* gene, which encodes typhoid toxins by SPI-3, was identified in seven serovars: *S. Weltevreden*, *S. Indiana*, *S. Give*, *S.*

Johannesburg, *S. Chester*, *S. Mkamba*, and *S. Potsdam*. This gene is known to be associated with programmed cell death (both apoptosis and necrosis), but it has also been recorded in *S. Indiana*, in accordance with the findings in our study (Yan et al., 2022). The plasmid-borne virulence gene *spv* and the fimbriae-encoding plasmid gene cluster *pefABCD* were present in three serovars: *S. Typhimurium*, *S. Weltevreden*, and *S. Mbandaka*. These two genes, *spv* and *pef*, played an important role in enabling *Salmonella* to survive, grow, and escape host immune response, and they have been found in *S. Typhimurium* (S. Fatima et al., 2023; Yan et al., 2022). This study underscores the diversity of virulent genes with varying functions that aid in bacterial survival and infection, as well as the strain's evolutionary adaptation in developing AMR.

Table 5. Virulence genes detection among identified *Salmonella* serovars

Functional Groups	Virulence genes (No. of isolates)
Effector delivery system	T3SS-SPI-1: <i>avrA</i> (68), <i>invABCEFGHIJ</i> (75), <i>orgABC</i> (74), <i>prgHIJK</i> (74), <i>sip/sspABC</i> (74), <i>sipD</i> (75), <i>slrP</i> (72), <i>sopADE2</i> (74), <i>sopB/sigD</i> (74), <i>spaOPQRS</i> (75), <i>sptP</i> (73), <i>sspH1</i> (11), <i>sicAP</i> (75) T3SS-SPI-2: <i>gogB</i> (15), <i>PipB & PipB2</i> (74), <i>sifAB</i> (74), <i>spiC/ssdB</i> (72), <i>sseABCDEFGJK1&2L</i> (75), <i>sseI/srfH</i> (12), <i>sspH2</i> (50), <i>steC</i> (72), <i>ssa CDEGHIJKLMNOPQRSTUVWXYZ</i> (75), <i>sscAB</i> (74), <i>sopD2</i> (70), <i>spvC</i> (4)
Adherence	<i>csgABCD</i> (75), <i>csgEFG</i> (38), <i>faeCDE</i> (30), <i>fimCDFHI</i> (75), <i>ipfABCDE</i> (49), <i>ompA</i> (75), <i>sinH</i> (72), <i>shdA</i> (12), <i>steABC</i> (73), <i>ratB</i> (44), <i>misL</i> (71), <i>ybtPQ</i> (3)
Immune modulation	<i>tcpC</i> (2), <i>rck</i> (4), <i>cpsH</i> (2)
Metabolic factors	<i>entAB</i> (73), <i>fepCDG</i> (37)
Stress survival	<i>sodCI</i> (14),
Exotoxin	<i>spvB</i> (4), <i>cdtB</i> (10)
Plasmid virulence gene	<i>spvR</i> (4)
Antimicrobial peptide resistance protein	<i>mig-14</i> (69)
Others	<i>fyuA</i> (3), <i>yagZ/ecpA</i> (2), <i>tcpC</i> (2), <i>ykgK/ecpR</i> (2), <i>bopD</i> (1), <i>yagV/ecpE</i> (1), <i>yagW/ecpD</i> (1), <i>yagX/ecpC</i> (1), <i>yagY/ecpB</i> (1), <i>ybt AESTUX</i> (3)

4.4.3 *Salmonella* pathogenicity islands

A total of 12 SPIs were detected across the 75 isolates of 32 *Salmonella* serovars, including C63PI, CS54_island, SPI-1 through SPI-5, SPI-8, SPI-9,

SPI-12 to SPI-14 (Figure 9). SPI-1 to SPI-3, SPI-5, and SPI-9 were detected across all serovars, whereas SPI-12 was only found in a single *S. Braenderup* isolate. In *Salmonella* isolates recovered from duck carcasses in Hanoi, Vietnam, SPI-1 to SPI-3 were detected in all *Salmonella* isolates, indicating widespread distribution (Nguyen et al., 2023).

Of the 32 serovars, 11 distinct SPI patterns were identified: pattern 1 (8 SPIs, serovar n=4), pattern 2 (10 SPIs, serovar n=8), pattern 3 (9 SPIs, serovar n=7), pattern 4 (8 SPIs, serovar n=2), pattern 5 (6 SPIs, serovar n=2), pattern 6 (7 SPIs, serovar n=2), pattern 7 (9 SPIs, serovar n=1), pattern 8 (10 SPIs, serovar n=1), pattern 9 (11 SPIs, serovar n=1), pattern 10 (8 SPIs, serovar n=1), and pattern 11 (9 SPIs, serovar n=1). For comparison, a study on *Salmonella* isolates from dogs and cats reported the presence of SPI-1 to SPI-5 in all isolates, with four distinct SPI patterns observed (Puangseree et al., 2025). Recent studies have described distinct SPI patterns in specific *Salmonella* serovars. Fatima et al. (2023) identified three such patterns: one pattern, composed of C63PI, CS54_island, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13, and SPI-14, was observed in *S. Typhimurium* and corresponds to pattern 2 in our study. The other two patterns detected in *S. Typhi* (SPI-1, SPI-2, SPI-3, SPI-5, and SPI-9) and (SPI-1, SPI-2, SPI-3, SPI-5, SPI-7, and SPI-9), were not identified in the present work. A separate study exhibited 7 SPI patterns for *S. Muenster*, and 1 pattern for *S. Kentucky* (Nguyen et al., 2023).

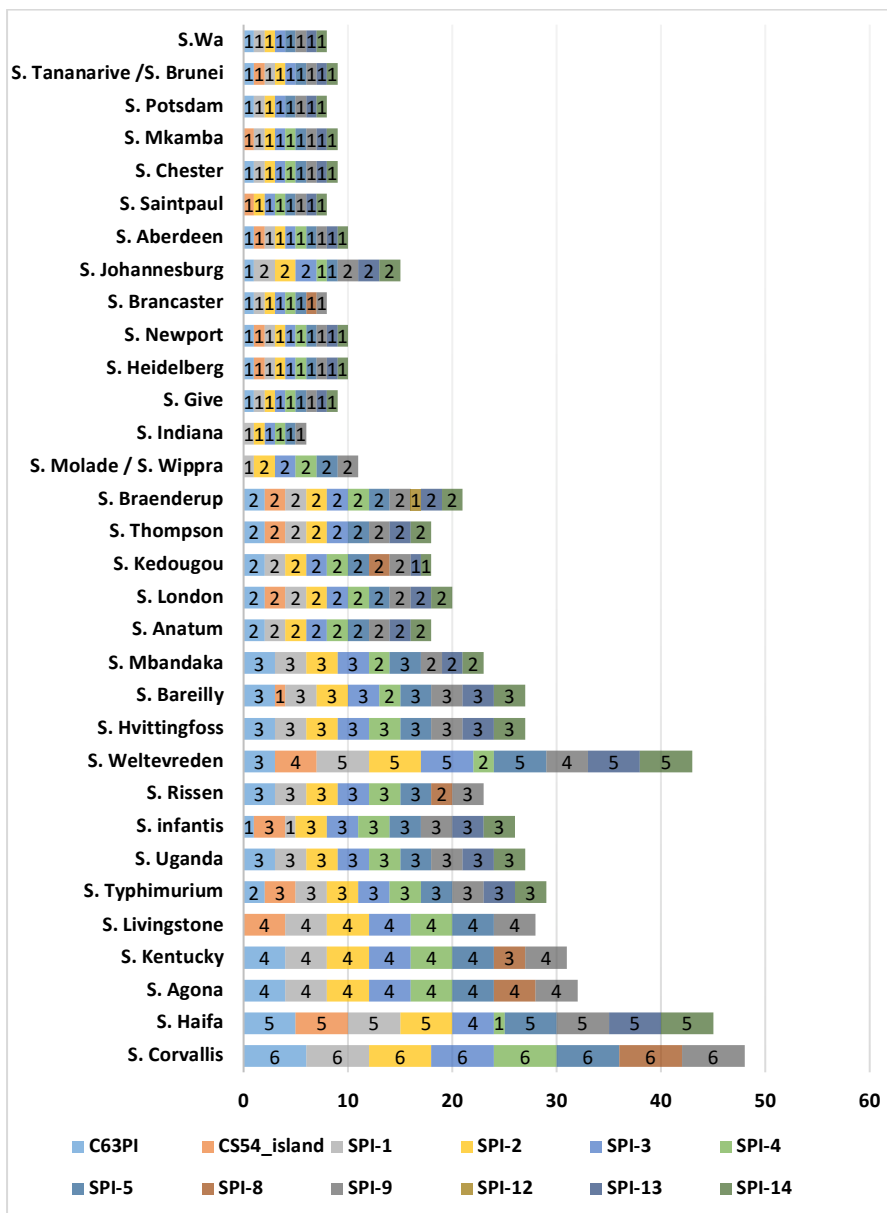


Figure 9. The frequency of the *Salmonella* pathogenicity islands (SPIs) found on different *Salmonella* serovars (n = 75 isolates) isolated from different food commodities in this study.

5. Conclusions

Food safety in Cambodia continues to pose significant public health challenges, particularly in regard to foodborne pathogens such as *Salmonella* spp. and their associated antimicrobial resistance (AMR). *Salmonella* has multiple opportunities to spread along the food chain, commencing from primary sources of contamination in livestock and agricultural production, before moving through markets and ultimately leading to human infection. The present study provides baseline data on foodborne pathogen contamination in fresh food products in Cambodia, with a focus on the prevalence of *Salmonella* spp. and its AMR-related genetic traits. The main conclusions are:

- The overall prevalence of *Salmonella* spp. was 48% (138/285 samples), with higher detection rates in meat (71%) and seafood/fish (64%) than in vegetables (33%). Six common serovars, namely *S. Corvallis*, *S. Haifa*, *S. Weltevreden*, *S. Agona*, *S. Kentucky*, and *S. Livingstone*, were identified. These results confirm that fresh food products in local markets and farms are major sources of *Salmonella* transmission.
- Cross-contamination risks were observed in both markets and farms, and are likely associated with poor hygiene and sanitation, as well as improper food handling, storage, and preservation practices.
- Phenotypic and genotypic analyses revealed a high prevalence of AMR among *Salmonella* isolates, along with a wide variety of AMR-associated genes, highlighting the extensive occurrence of resistant strains in fresh food products.
- Genetic characterization across *Salmonella* serovars demonstrated considerable diversity, including plasmids, virulence-associated genes, and pathogenicity islands, which may contribute to the emergence and persistence of AMR strains throughout the food chain.

Overall, this thesis demonstrated that *Salmonella* isolated from fresh food products exhibit high virulence genes, which enable them to become high-virulence variants with varying abilities to cause human infections. Thus, controlling the spread of foodborne salmonellosis in Cambodia requires a

comprehensive strategy that combines targeted food safety interventions with strong antimicrobial stewardship measures.

6. Future consideration

The present findings provide valuable insights into the critical role that the food chain plays in disseminating resistant bacteria from livestock and agricultural environments to humans. Understanding the pathways of AMR transmission and identifying critical control points are essential for developing effective intervention strategies.

To gain a clearer understanding of the AMR transmission pathway, an extended study is needed to investigate how *Salmonella* develops AMR, whether through genetic mutation, via genetic shift, or by transfer from other microorganisms. Thus, the identification of the abundance of microbial diversity in the food chain and its genetic characterization should be further performed, focusing on how resistance genes and other virulence genes are transferred from one bacterium to another. Moreover, future research should focus on increasing the sample types and sample size, for instance, by extending the number of food samples in more local markets with seasoning monitoring and increasing the number of local vegetable and livestock farms to trace the source of food contamination. These results can inform policymakers, as well as the relevant ministry, of further action plans and a prevention and control strategy for foodborne pathogens.

Regarding the high prevalence of *Salmonella* and its AMR, combined with the cross-contamination observation from this study's sampling site, further action should be considered. To enhance hygiene, sanitation, and food handling practices, comprehensive training should be provided at both market and farm levels. This training should encompass proper handwashing techniques, effective environmental sanitation, waste management, safe food storage practices, and an awareness of foodborne pathogens and their AMR among local farmers, vendors, and household consumers. Moreover, the AMR education intervention, along with the exploratory and scientific assessments, should be considered to understand the behavior, awareness, and willingness of Cambodians, especially high school and university students, to improve public knowledge of the proper use of antibiotics and to combat the spread of AMR.

Furthermore, to improve food safety and public health response, expanding laboratory capacity and strengthening monitoring systems should be considered. This includes expanding standard laboratory facilities beyond urban areas to rural regions of Cambodia and ensuring that they are staffed

with trained specialists. Doing so will enable faster and more accurate reporting of foodborne outbreaks and other harmful organisms, allowing for quicker interventions. Crucially, these facilities will support both research and medical care, particularly in communities with limited access to health services.

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Popular science summary

In Cambodia, most individuals purchase their fresh food for daily household meals from local or informal markets, which serve around 90% of urban households and 50% of rural households. The rest rely on home gardens and mobile food vendors. However, because these local markets often lack standardized regulations and routines for maintaining hygienic food handling practices, food safety remains a major public health concern, particularly regarding the risk of infections caused by foodborne pathogens. Previous studies in Cambodia have identified bacterial pathogens such as *Campylobacter*, *Salmonella* spp., *E. coli*, and *Listeria* spp. in the food chain. These pathogens are responsible for a high level of foodborne illnesses in Cambodia. Thus, further research is necessary to gain a better understanding of their prevalence, track courses of transmission, and implement preventive measures to restrict the spread of these pathogens. *Salmonella* spp. is particularly important, and previous studies have shown that more than 50% of fresh food products sold at local markets in Cambodia are contaminated by *Salmonella*. However, the mechanisms underlying *Salmonella* transmission throughout the food chain, as well as the development of its high virulence traits, are poorly understood. In this thesis, a systematic approach was used to determine the prevalence of *Salmonella* from various food items commonly consumed in Cambodia, followed by analyses of serovar distribution, antimicrobial resistance (AMR) at both phenotypic and genotypic levels, and the characterization of genetic elements such as plasmids, virulence genes, and *Salmonella* pathogenicity islands (SPIs) within the identified *Salmonella* serovars. The research was carried out in local markets and farms located in Phnom Penh City and Kandal Province, Cambodia.

A total of 285 samples, composed of meat, seafood/fish, and vegetables, were collected from five local markets in Phnom Penh. Vegetables were also obtained directly from nearby farms that supplied the markets. The overall contamination rate was notably high (48%), but meat samples demonstrated the highest level (71%). The high prevalence is likely due to poor hygiene and sanitation practices during handling, storage, and preservation at both farm and market levels. AMR was also found to be substantial, with 71% of isolates being resistant to at least one antibiotic, and 39% exhibiting

multidrug resistance. Whole-genome sequencing (WGS) was performed on isolates possessing high-quality DNA, with a primary focus on AMR-positive isolates, while non-AMR isolates were used as reference strains. WGS identified 32 serovars, with six being the most common, namely *S. Corvallis*, *S. Haifa*, *S. Weltevreden*, *S. Agona*, *S. Kentucky*, and *S. Livingstone*. Further genetic characterization revealed the presence of antimicrobial resistance genes (ARGs), plasmids, virulence genes, and SPIs. Overall, WGS analysis identified 144 ARGs across 81 WGS *Salmonella* isolates. Substantial genetic analysis also revealed the identification of 22 plasmids, 150 virulence genes, and 12 SPIs among 32 *Salmonella* serovars. These results demonstrate substantial genetic diversity among *Salmonella* strains, which may suggest a strong link between genetic variation and the high prevalence of AMR strains occurring within the local food supply chain in Cambodia.

Populärvetenskaplig sammanfattning

I Kambodja köper de flesta människor sin mat dagligen från lokala eller informella marknader, vilka försörjer cirka 90 % av hushållen i städerna och 50 % på landsbygden. Resterande hushåll producerar sin egen mat, samt köper från mobila livsmedelsförsäljare. Eftersom lokala marknader ofta är oreglerade och saknar rutiner för att upprätthålla god livsmedelshygien, utgör livsmedelssäkerhet ett betydande folkhälsoproblem, särskilt med avseende på risken för infektioner orsakade av livsmedelsburna patogener. Tidigare studier i Kambodja har identifierat bakteriella patogener såsom *Campylobacter*, *Salmonella* spp., *E. coli* och *Listeria* spp i livsmedelskedjan. Dessa patogener bidrar i hög grad till livsmedelsburna sjukdomar i Kambodja. Ytterligare forskning är därför nödvändig för att få en bättre förståelse av deras förekomst, smittvägar och för att genomföra åtgärder för att begränsa spridning av dessa patogener. *Salmonella* spp. är av speciell betydelse och tidigare studier har visat att mer än 50 % av färska livsmedel som säljs på lokala marknader är kontaminerade av bakterien. De bakomliggande mekanismerna för bakteriens spridning i livsmedelskedjan, samt utvecklingen av dess virulensegenskaper, är emellertid ännu otillräckligt klarlagda. I denna avhandling fastställdes förekomsten av *Salmonella* från olika livsmedel som ofta konsumeras i Kambodja, följt av analyser av vilka serovarer som förekom, antimikrobiell resistens (AMR) på både fenotypisk och genotypisk nivå, samt karakterisering av genetiska element såsom plasmider, virulensgener och *Salmonella* patogenicitetsöar (SPIs) hos de identifierade serovarna. Studierna i avhandlingen genomfördes på lokala marknader och gårdar belägna i Phnom Penh och Kandal-provinsen i Kambodja.

Totalt samlades 285 prover in, bestående av kött, skaldjur, fisk och grönsaker, från fem lokala marknader i Phnom Penh. Grönsaker samlades även in från närliggande gårdar som levererar till marknaderna. Det var en hög generell förekomst (48 %) av *Salmonella* spp. i provtagna livsmedel och den högsta nivån noterades hos kött (71%). Den höga förekomsten beror sannolikt på bristande hygien och rutiner vid hantering, lagring och förvaring både på gårds- och marknadsnivå. Det var också en hög förekomst av AMR, 71 % av isolaten var resistenta mot minst ett antibiotikum och 39 % uppvisade multiresistens. Helgenomsekvensering (WGS) utfördes främst på

AMR-positiva isolat som hade högkvalitativt DNA, medan icke-AMR-isolat användes som referensstammar. Genom WGS identifierades 32 serovarer, varav de sex vanligaste var: *S. Corvallis*, *S. Haifa*, *S. Weltevreden*, *S. Agona*, *S. Kentucky* och *S. Livingstone*. Ytterligare genetisk karakterisering visade på förekomst av gener som leder till antimikrobiell resistens (ARGs), plasmider, virulensgener och SPIs. Totalt identifierade WGS-analysen 144 ARGs bland 81 *Salmonella*-isolat. De genetiska analyserna visade även att det fanns 22 olika plasmider, 150 virulensgener och 12 SPIs bland de 32 *Salmonella* serovarer. Dessa resultat visar på en betydande genetisk mångfald bland *Salmonella*-stammar, vilket tyder på ett starkt samband mellan genetisk variation och den höga förekomsten av AMR-stammar i den lokala livsmedelskedjan i Kambodja.

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Prevalence of *Salmonella* spp. in meat, seafood, and leafy green vegetables from local markets and vegetable farms in Phnom Penh, Cambodia

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ABSTRACT

Salmonella is a major bacterial concern for public health globally. Although there are limited documentation on the prevalence of *Salmonella* species in Cambodia's food chain, some reports indicate that salmonellosis is a severe gastrointestinal infection in its population and especially in children. To investigate the presence of *Salmonella* spp., 285 food samples (75 meat, 50 seafood, and 160 leafy green vegetable samples) were randomly collected from various local markets in Phnom Penh capital and nearby farms in Cambodia. Concurrently, field observations were conducted to collect data on food hygiene and practices among the relevant actors. All food samples were analyzed using bacterial culture and plate counts, and the findings were confirmed serially with biochemical, serological, and PCR tests. The observational data on food hygiene and practices from farm to market revealed that the spread of *Salmonella* in the food-value chain from farm to market could pose health risks to consumers. The overall prevalence of *Salmonella* spp. was 48.4% (138/285), while the prevalence in meat, seafood, and vegetables was 71% (53/75), 64% (32/50), and 33% (53/160), respectively. Mean *Salmonella* plate count ranged from 1.2 to 7.40 log₁₀ CFU/g, and there was no significant difference in bacterial counts between meat, seafood, and vegetable samples ($p > 0.05$). The most common serogroups among the isolated *Salmonella* spp. were B and C. These results suggest that a large proportion of meat, seafood, and vegetable products sold at local markets in Phnom Penh are contaminated with *Salmonella* spp. This is likely linked to inadequate hygiene and sanitation practices, including handling, storage, and preservation conditions. Observations on farms suggested that the prevalence of *Salmonella* in vegetables sold at the market could be linked to contamination relating to agricultural practices. Thus, controlling the spread of foodborne salmonellosis through the food-value chain from farms and retailers to consumers is warranted to enhance food safety in Cambodia.

1. Introduction

Foodborne illnesses are a significant public health concern and may result from improper food handling, inadequate cooking or storage, and use of water contaminated with pathogens such as bacteria, viruses, and parasites (St Amand et al., 2017). It has been estimated that foodborne diarrheal illnesses cause at least 230,000 deaths worldwide, with non-typhoidal *Salmonella enterica* being reported to be one of the four leading foodborne diarrheal agents, accounting for approximately 59,000 global deaths (Havelaar et al., 2015; WHO, 2018).

Salmonella is a group of Gram-negative rod-shaped bacteria belonging to the *Enterobacteriaceae* family and consists of two species known as *Salmonella bongori* and *S. enterica* (Issenhuth-Jeanjean et al., 2014; Reeves et al., 1989). Based on three distinct surface structures, i.e., lipopolysaccharides (LPS), flagella, and capsular polysaccharides, approximately 2600 serovars of *S. enterica* have been distinguished (Ferrari et al., 2019; Popoff et al., 2004). *Salmonella* species can also be categorized based on their capacity to induce particular medical conditions in humans, distinguishing between typhoidal strains that affect only humans and non-typhoidal (NTS) strains that affect both humans

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and animals (Diaz et al., 2022; Ferrari et al., 2019).

Salmonella can spread to humans through contaminated foods such as fresh meat, vegetables, and dairy products (Huoy et al., 2014; Abatcha et al., 2018; Rönnqvist et al., 2018), through environmental sources, including water, (St Amand et al., 2017; Gu et al., 2019), and through contact with human or animal feces (Penakalapati et al., 2017). Several studies have shown that *Salmonella* contamination can occur at several points in the food production chain (Arnold et al., 2010; Lassnig et al., 2012; Bonardi, 2017; Ehuwa et al., 2021). *Salmonella* is of particular importance in Southeast Asian countries, such as Cambodia. According to recent estimates by the Global Burden of Disease, more than 24,000 typhoid cases (123 cases per 100,000) resulting in around 300 deaths occurred in Cambodia during 2019 (Havelaar et al., 2015). Studies in other Southeast Asian countries have found a high prevalence of NTS *Salmonella* contamination in fresh meat and vegetables at farm and market level (Lettini et al., 2016; Meunsene et al., 2021; Patra et al., 2021; Vidayanti et al., 2021).

Fresh meats and vegetables are popular in the Cambodian diet and can be important sources of *Salmonella* spp. infection. Cambodia's food production chains and markets are informal, with opportunities for bacterial cross-contamination during transportation, distribution, and sale (Chhean et al., 2004; Mosimann et al., 2023). Currently, there are three forms of markets in Cambodia: i) wholesale markets (meat and vegetables are primarily sold in large quantities, while fresh products are distributed to other vendors or small open markets); ii) retail markets (selling foodstuffs or other items directly to end users); and iii) superstore/close markets (supermarkets and private stores selling self-claimed organic produce). There are few published reports on the occurrence of *Salmonella* spp. along the food chain in Cambodia, but previous reports indicate that salmonellosis is a severe gastrointestinal illness in humans, particularly children (Emary et al., 2012; Wijedoru et al., 2012; Chheng et al., 2013; Kheng et al., 2020). A recent study found that the prevalence of *Salmonella* spp. in chicken meat and pork collected at Cambodian markets was 42% (Rortana et al., 2021). It has been found that the prevalence of *Salmonella* spp. in fresh lettuce is higher during the dry season (56.5 %) compared with the rainy season (15.4%) (Desiree et al., 2021).

More science-based data are needed to understand microbial contamination mechanisms for different food types at markets, farms, and slaughterhouses and to enable mitigation and preventive methods to be implemented. This study aimed to investigate the prevalence of *Salmonella* spp. in meat, seafood, and leafy green vegetables collected from various local markets in Phnom Penh and from farms located close to Phnom Penh, Cambodia, and to investigate food safety procedures at markets that might pose risks of *Salmonella* cross-contamination.

2. Materials and methods

2.1. Study area

Cambodia is located in Southeastern Asia, bordering Laos, Vietnam, Thailand, and the Gulf of Thailand. The total land area is 181,035 square kilometers, with a population of around 14.6 million people. Cambodia's climate is dominated by the tropical monsoon, with temperatures ranging from 21 to 35 °C and with peaks of up to 40 °C during April. There are two main seasons: the rainy season (May to November) and the dry season (December to April) (NAP-GSP, 2022). Cambodia has 24 provinces, and the central capital is Phnom Penh (NIS, 2019). Phnom Penh acts as a hub for receiving and distributing fresh food from different locations in Cambodia, e.g., 98% of vegetables (choysum, lettuce, and yard long bean) from Kandal province were supplied to local markets in Phnom Penh in 2002 and 2003 (Chhean et al., 2004). Most Cambodians buy groceries, fresh meats, and vegetables at local markets, which can be either formal or informal. The informal markets are unregulated and consist of a decentralized community of producers, distributors, and sellers (Desiree et al., 2021). Phnom Penh City and Kandal

Province were selected as study areas for our analysis, enabling easy access to the laboratory.

2.2. Study design and data collection

The design of the present study was a cross-sectional study which was conducted between November 2020 and November 2021. A previous study in Cambodia showed *Salmonella* prevalence in poultry samples of 88 % (Lay et al., 2011), a value which we used for calculating the number of samples required in the present study. The aim was to include 250 samples from different food categories collected from the selected markets (see below) to give an estimated a prevalence of 80%, with a confidence level of 95% and precision of 5%.

Food samples were collected from one wholesale market (Market 1), three retail markets (Markets 2, 3 and 4), and superstore/closed markets (Market 5, including organic shops and supermarkets) in Phnom Penh Capital (Fig. 1). The number of vendors from each market depended on their availabilities of the sample types included in the study. Selected vendors could provide more than one sample type, but each sample type was collected from each individual vendor separately. The sampling teams decided the vendors to include to ensure equal distribution of the vendors in the markets. Twenty-eight vendors selected from Market 1, 26 from Market 2, 24 from Market 3, 19 from Market 4 were included in our sampling. To extend the understanding of the *Salmonella* contamination for common market forms across Cambodia, samples were collected from 11 organic shop and supermarkets to represent Market 5.

We planned to include 50 samples per market (25 leafy vegetable samples, 15 meat samples (pork, beef and chicken), and 10 from seafood samples), and 15 vegetable samples per farm (Table 1). Two vegetable farms located in Kandal province, known for supplying vegetables to the markets in the capital Phnom Penh, were also sampled (Fig. 1).

All samples were randomly collected. Each sample consisted of 500g and were collected aseptically using sterile gloves. Each sample package was cleaned with 70% ethanol to prevent cross-contamination. All samples were stored in a cool box with ice packs for a maximum of 5 h before analysis. Samples from vendors were collected within the shelf life of up to seven days, counted from the harvest date for vegetables and seafood and one day for meat from the slaughterhouse. All samples were given unique identification numbers and were placed in secure sterile resealable plastic bags in a cool box containing ice packs for immediate transport to the laboratory. Samples that had been improperly packaged or visibly damaged were discarded before analysis. All samples were brought to the Microbiology Laboratory at the Department of Food Chemistry, Faculty of Science and Technology, International University (IU), Phnom Penh, Cambodia.

Epidemiological data related to food hygiene and practices at the selected farms and markets were collected through observations and interviews during sampling, using pre-made checklists at the included markets and farms. Training on sample collection and on conducting observations and interviews was provided to the research team (third- and fourth-year BSc students from the Royal University of Phnom Penh (RUPP) and IU) before the start of the study. The observational part of the study focused on measuring ambient temperature, noting the storage containers used, checking whether the vendors or farmers used personal protection equipment (PPE), and recording other potential risk factors for *Salmonella* transmission related to food-contact surface areas. The interviews with vendors and farmers focused on how the food was processed and handled at markets and on farms, as well as on the transportation of the food. All data were recorded using the pre-made checklists. The research team informed the vendors and farmers about the study and said that participation would be voluntary and anonymous. They also asked for oral consent to participate in the study. At the end of each day, data were transferred to Microsoft Excel for further analysis and checked for accuracy. The study was approved by the Royal University of Phnom Penh under the grant agreement between Sweden and RUPP (19000439) on January 22, 2019.

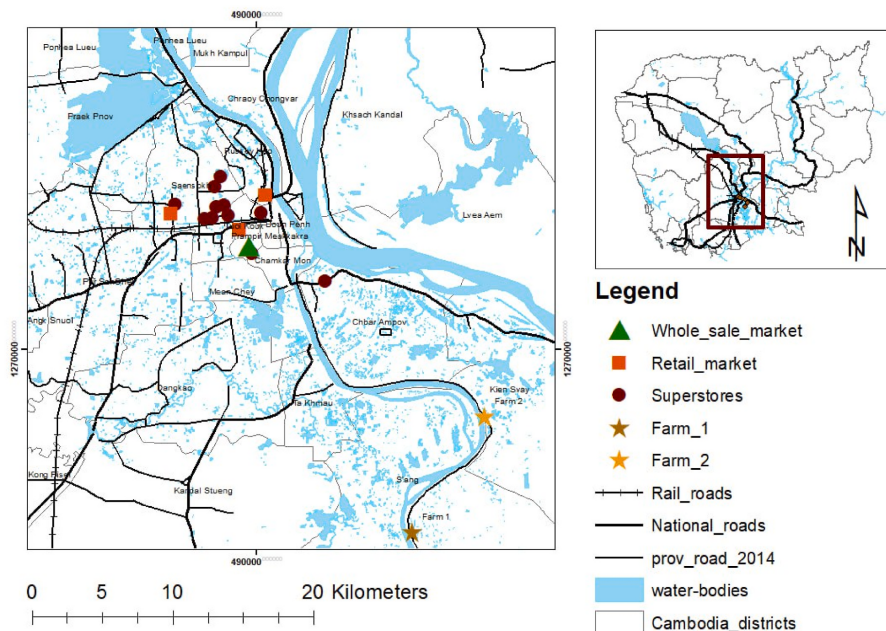


Fig. 1. Map of sampling sites.

Table 1

Food samples collected from markets and vegetable farms in Cambodia and analyzed to determine the prevalence of *Salmonella* spp.

Sample types		Scientific name	No. of Samples Collected							Total no. of samples
			Market 1	Market 2	Market 3	Market 4	Market 5	Farm 1	Farm 2	
Meat	Beef	<i>Bos indicus</i>	5	5	5	5	5	–	–	25
	Chicken	<i>Gallus gallus domesticus</i>	5	5	5	5	5	–	–	25
	Pork	<i>Sus scrofa domesticus</i>	5	5	5	5	5	–	–	25
Seafood	Fish	<i>Channidae</i> (Snake Head Fish)	5	5	5	5	5	–	–	25
	Seafood	<i>Caridean shrimp</i>	5	5	5	5	5	–	–	25
Vegetable	Bok Choy	<i>Brassica rapa</i> subsp. <i>chinensis</i>	5	5	5	5	5	5	5	35
	Salad	<i>Lactuca sativa</i> var. <i>crispa</i>	5	5	5	5	5	5	5	35
	White cabbage	<i>Brassica oleracea</i> var. <i>capitata</i> f. <i>alba</i>	5	5	5	5	5	5	–	30
	Water morning glory	<i>Ipomoea aquatica</i>	5	5	5	5	5	5	5	35
	Curly cabbage	<i>Brassica oleracea</i> var. <i>capitata</i> f. <i>sabauda</i>	5	5	5	5	5	–	–	25
Total no. of samples			50	50	50	50	50	20	15	285

2.3. Microbiological analysis

In all microbiological analysis, a negative control (*E. coli* ATCC 25922) and a positive control (*Salmonella enterica* subspecies *enterica* serotype Typhimurium ATCC 14028) were used.

2.3.1. Bacterial isolation

Salmonella was isolated according to ISO method 6579–1:2017. In brief, meat, seafood, and vegetable samples were cut into small pieces using a sterile knife, and 25 g of sample were mixed thoroughly with 225 mL buffer peptone water (BPW, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Then, 1 mL was transferred to a 1.5 mL centrifuge tube for serial dilution and plating on *Salmonella*-Shigella

agar (SS, HiMedia, Maharashtra, India) for *Salmonella* quantification (Malorny et al., 2008). The plates were incubated at 37 °C for 18–24 h. Colorless colonies with a black center were presumed to be *Salmonella*. Colonies typical of *Salmonella* were counted all over the entire plates, if less than 300 colonies. The total *Salmonella* concentration (CFU/g) was calculated using actual colony counts multiplied by the dilution factor, then divided by the volume of the sample used.

The remaining sample mixture was incubated in a shaker (KS 4000 i control, IKA-Werke GmbH & Co.KG, Staufen, Germany) at 37 °C for 18 h. The pre-enrichment culture was then incubated for 18–24 h in two selective enrichment media, Muller-Kauffmann Tetrathionate Novobiocin broth (MKTn, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and Rappaport Vassiliadis Soya Peptone broth (RVS, Sigma-

Aldrich Chemie GmbH, Taufkirchen, Germany), at 37 °C and 42 °C, respectively. Each sub-culture was cultured on xylose-lysine-desoxycholate agar (XLD, HiMedia, Maharashtra, India), and Brilliant Green Agar (BGA, Sigma- Aldrich Chemie GmbH, Taufkirchen, Germany) at 37 °C for 24 h and then checked for typical *Salmonella* colonies. Each sample was analyzed in triplicate.

2.3.2. Bacterial confirmation

2.3.2.1. *Morphological and serological confirmation.* Three to five typical colonies were chosen from each XLD and BGA plate. Selected colonies were confirmed by Gram staining with microscopic morphology identification. Colonies with red/pink color containing rod-shaped bacteria were considered suspected *Salmonella* spp. Presumptive *Salmonella* isolates were confirmed using the Latex slide agglutination commercial LK02-HiSalmonella™ Latex test kit (HiMedia, Maharashtra, India). First, autoagglutination was tested, and any colony material from isolates that did not show autoagglutination was picked from XLD or BGA agar, mixed with 20 µL Latex reagents and observed for agglutination in accordance with the kit instruction. The *Salmonella* Sero-Quick Group kit (SSI Diagnostica A/S, Hillerød, Denmark) was used to identify the most common *Salmonella* serogroups.

2.3.2.2. *Biochemical confirmation.* Once a pure culture was obtained, each isolate was tested with the KBM002 Himotility™ Biochemical kits for *Salmonella* (HiMedia, Maharashtra, India). A single isolated colony was inoculated in 5 mL of Brain Heart Infusion broth (BHI, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and incubated at 37 °C for 4 h until turbidity of more than 0.1 OD at 620 nm was measured with a UV Spectrophotometer (UV-3000 spectrophotometer, LC-Instrument). A loopful of mixed culture was then inoculated into each well, except the second well, for detecting the motility of the strain. After incubation at 37 °C for 24–48h, the color change was recorded by comparing the result with the standard data sheet provided with the kit.

2.3.2.3. *PCR-based confirmation.* Genomic DNA from each isolate was prepared using a QIAamp blood Mini kit (Hilden, Germany) following the manufacturer’s instructions. To confirm presence of the *Salmonella* *invA* gene, the commercial Primer set SIN-1, SIN-2 (Takara Bio Europe SE, Göteborg, Sweden) was selected with amplified fragment 378bp, with *S. Typhimurium* ATCC14028 as the positive control. PCR amplification was performed in a final volume of 25 µL in a thermocycler with conditions described in the manufacturer’s manual. PCR Master Mix reagent (Thermo Fisher Scientific, Vilnius, Lithuania) was used for PCR amplification. A 12.5 µL aliquot of PCR Green Master Mix was dispensed into each PCR tube with 9.5 µL deionized distilled water and 10 pmol/µL of each forward and reverse primer (0.5 µL of each primer per reaction). Template DNA (2 µL) was added before loading into the SimpliAmp™ Thermal Cycler. The PCR amplification steps were performed as following the instructions in the primer set: 1 min initial denaturation at 94 °C, followed by 35 cycles (94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min), and final extension at 72 °C for 10 min. The PCR products were run through 1.5 % agarose gel in 1X TAE buffer at a constant voltage of 85 V for 80 min. As size marker, 1 µL of 1 kb ladder plus was used, and 5 µL gel red staining solution was added per 50 mL agarose gel. The PCR product was visualized as a single fluorescent band using Chemidoc Touch images (Bio-Rad, Image Lab Gel Doc™ EZ system).

2.4. Statistical analysis

IBM SPSS statistic version 27 was used to perform statistical analysis. All bacterial plate counts were converted into log₁₀ CFU/g. A one-way ANOVA test was performed to check for significant differences in mean *Salmonella* spp. plate count on meat, seafood, and vegetable samples. The prevalence of *Salmonella* spp. in different sample types and

different markets was compared using the Chi-square (χ^2) test, with $P \leq 0.05$ considered to represent a statistically significant difference.

3. Results

In total, 285 fresh vegetables and meat samples were collected at local markets in Phnom Penh city and from farms located in Kandal Province (Fig. 1). Of these, 125 were meat (n = 75) and seafood (n = 50) samples from local markets, and 160 were leafy green vegetables from local markets and farms (Table 1).

3.1. Prevalence of *Salmonella* spp. in food samples

Based on serial testing of each sample, from conventional culture followed by biochemical and serological tests and finally with PCR confirmation with *invA* gene detection, 138 (48.4%) of the 285 samples tested positive for *Salmonella* spp. These comprised 53 (71%) meat and 32 (64%) seafood samples collected from local markets and 53 (33%) vegetable samples from local markets and farms (Table 2). At the market level, combined *Salmonella* spp. prevalence in meat, seafood, and vegetables from the wholesale market (78%) was significantly higher than that recorded at retail markets (Markets 2, 3, and 4, 43%) and the superstores/closed market (Market 5, 46%), ($P < 0.001$). There was no difference in the prevalence of *Salmonella* spp. between vegetables collected at the different markets and farms (Fig. 2).

The *Salmonella* spp. plate count results for meat, seafood, and vegetable samples from the selected markets are presented in Fig. 2. The mean total count of *Salmonella* spp. on meat samples ranged from 1.8 to 3.4 log₁₀ CFU/g, while that on seafood samples ranged from 1.8 to 7.4 log₁₀ CFU/g and that on fresh vegetables ranged from 1.2 to 4.1 log₁₀ CFU/g. There was no significant difference in total *Salmonella* spp. counts between meat, seafood, and vegetable samples. On the other hand, there were significant differences in total *Salmonella* spp. count between vegetable samples taken from Market 1, Market 2, Market 3, Market 4, and Market 5 ($p < 0.05$) (Fig. 2).

According to the serogroup results, serogroups B and C were the most common serogroups among the positive samples accounting for 20% (28 out of 138 isolates) and 33% (45 out of 138 isolates), respectively (Fig. 3). Serogroup C was present in all food commodities, while serogroup B was not found in white cabbage samples.

3.2. Observed food safety risk

Data on food hygiene and practices were collected from 27/28

Table 2
Proportion of meat and vegetable samples from selected markets and farms in Cambodia testing positive for *Salmonella* spp.

Sampling region		Meat		Vegetable (pos/ total)	No. of positives	Prevalence (%)
		Meat (pos/ total)	Seafood (pos/ total)			
Market	Market 1	15/15	10/10	14/25	39/50	78.0
	Market 2	10/15	2/10	6/25	18/50	36.0
	Market 3	8/15	1/10	4/25	13/50	26.0
	Market 4	13/15	9/10	12/25	34/50	68.0
	Market 5	7/15	10/10	6/25	23/50	46.0
Farm	Farm 1	–	–	4/20	4/20	20.0
	Farm 2	–	–	7/15	7/15	46.7
Total		53/75	32/50	53/160	138/285	48.4

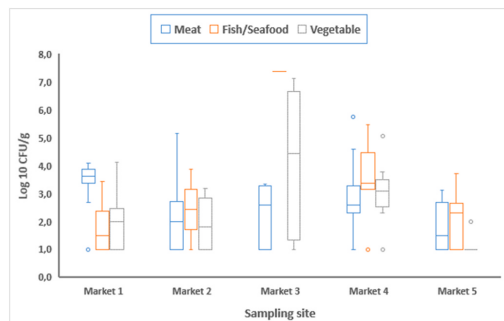


Fig. 2. Boxplot of Mean total plate count of *Salmonella* spp. different meat, seafood, and vegetable samples collected from selected markets in Cambodia.

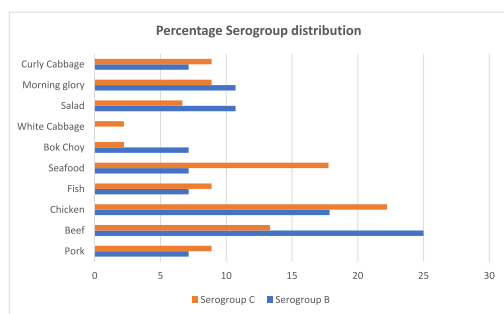


Fig. 3. Percentage of serogroup distribution among food commodities.

vendors from Market 1, 25/26 from Market 2, 18/24 from Market 3, 19/19 from Market 4, and 11/11 from Market 5. Farm observation data were collected from both included farms. The observation of vegetable farms showed that the actual temperature ranged between 30 °C and 31 °C. Fresh vegetables were kept directly on the ground after harvest before being packed in plastic bags for transport to market. There was no PPE used during vegetable harvesting, with only 20% (1 out of 5 farms) of farm 1 and 33% (1 out of 3 farms) of farm 2 rinsed the vegetables using mainly pond or stream water before transport to the markets. Other observed risks for bacterial cross-contamination during vegetable growing and harvest were, e.g., wet ground with some animal feces-contaminated soil near the growing areas and visibly dirty irrigation water due to dirty pipes or unclear water sources (Supplement Table 1).

Risks relevant to food hygiene were also observed in the selected markets. Significant temperature variations between the selected markets ranged from 19 to 36 °C. Table 3 describes the details of the potential risk for the spread of *Salmonella* among those markets. Different storage containers were used, including wooden, metal, and plastic. Most vendors used steel plates (21%), plastic bags (21%), directly placed on wooden tables (17%), and plastic containers (15%). Still, proper shelves were limited in wholesale and retail markets, except in super-stores/closed markets, where they were stored on a proper shelf with cold storage conditions. The PPE investigation showed limited use among markets, with 27 % (27 out of 100 response records) using mask, gloves, and apron, while 21 % (21 out of 100 response records) using masks only. Moreover, the observed risk arising during food handling shows that 48% (48 out of 99 responses) rinsed vegetable samples using recirculated tap water, while only 5% (5 out of 99 responses) used

Table 3

Observed/reported potential food safety risks among local markets.

Risk categories	Risky practice	Number of Responses	Percentage (%)
Storage	Plastic bag	21/100	21
	Steel plate	21/100	21
	Directly on the table	17/100	17
	Plastic basket	15/100	15
	Wooden board	12/100	12
	Cold storage (fridge)	6/100	6
	Tent mat	4/100	4
	Proper shelf	2/100	2
	Cardboard	2/100	2
Rinsing	Rinsing with tap water	48/99	48
	No rinsing	43/99	43
	Soaking	5/99	5
	Rinsing with pond water	3/99	3
Personal Protection Equipment (PPE)	No PPE	49/100	49
	Mask, gloves, and apron	27/100	27
	Mask	21/100	21
	Mask and gloves	3/100	3
Transportation	Motorcycle	37/97	38
	Car	28/97	29
	Trailer	22/97	23
	Tuk Tuk	8/97	8
	Bicycle	2/97	2
Flies	Around food vendors	24/100	24
	Next to the food vendors	32/100	32
Food display	Meat kept close to vegetables	23/100	23
	Different food types in the same containers	16/100	16

*Note: PPE Including mask, gloves, apron, or other protection material.

soaking for dirt removal and keeping the vegetables looking fresh. Other cross-contamination factors were also observed at the markets, including 16% (16 out of 100 responses) mixing food types in the same storage container, 32% (32 out of 100 responses) showed food vendors close with dirty ground, and 24% (24 out of 100 response) revealed abundant flies around the food areas.

In addition, the food supply sources were recorded for local supplies from Kandal, Kampong Cham, Kampot, Mondolkiri, and Svay Reang Provinces. The farms near Phnom Penh supplied most of the leafy green vegetables sold at the markets. Various means of transportation were used to transport fresh products from farms to the market, such as motorcycles (37%), cars (28%), and trailers (22%). The length of the pre-processing period showed that transport of vegetables, fish, and seafood could take one to two days, depending on the distance to the market, while transport of meat samples took less than 5 h. Therefore, the processing and storage period for all food commodities ranged from 2 h up to two days (Supplement Table 2).

4. Discussion

Our analysis revealed a high prevalence of *Salmonella* spp. on fresh meat, seafood, and vegetable samples at both local market and farm level. In addition, observations and interview responses revealed food safety practices that could contribute to the spread of *Salmonella* from farm to market and further to consumer. These practices included, e.g., reuse of storage containers without cleaning, mixing of meat and vegetable products in the same storage container, inadequate rinsing, a long period from harvest to market, and poor hygiene in food court areas. This confirms findings in previous studies, that lack of sanitation and improper practices by both farmers and vendors increase the risk of microbial cross-contamination at the market level (Trongjit et al., 2017; Schwan et al., 2021). Schwan et al. (2021) also observed a high

prevalence of *S. enterica* on food contact surfaces, indicating that microbial cross-contamination can occur when the same food containers are used interchangeably for different food types. A study on risk factors associated with *Salmonella* in pork shops in Vietnam revealed several risk factors, including the presence of flies or insects and the use of cloth material (Dang-Xuan et al., 2019). Additionally, another study indicated that livestock sourced meat, such as pork, can become contaminated with *Salmonella* within 30 min to 6 h of exposure to low concentrations of *Salmonella* in a slaughterhouse environment (Hurd et al., 2001). Thus, various practice enhancements, such as improved hygiene measures in slaughterhouses and at the market level, could potentially reduce the risk of salmonellosis from meat consumption.

Our analysis showed that fresh meat sold at local markets in Phnom Penh was frequently contaminated (71%) with *Salmonella* spp., which is consistent with findings from previous studies in Cambodia. For instance, one study found 88.2% *Salmonella* spp. prevalence in poultry from markets in Phnom Penh (Lay et al., 2011), while another detected 42.1% *Salmonella* spp. prevalence in chicken and pork samples collected from local markets across 25 Cambodian provinces (Rortana et al., 2021). Our study also revealed higher prevalence of *Salmonella* contamination in meat samples than reported in neighboring countries. For example, a study in Malaysia detected *Salmonella* spp. in 21% of raw chicken meat samples at retail markets (Thung et al., 2016), while a study in Thailand found that 23% of raw food samples at retail were contaminated with *Salmonella* (Kong-Ngoen et al., 2022). We found that fresh seafood sold in local markets in Phnom Penh was also highly contaminated with *Salmonella* spp. (64%). In contrast, in a study in Bangkok around 36% of samples collected from seafood markets tested positive for *Salmonella* (Atwill and Jeamsripong, 2021). A previous study on *S. enterica* persistence revealed a survival time of up to 15 days in fish and shrimp stored in ice (Don et al., 2020). *Salmonella* contamination of seafood can occur in the natural aquatic environment, in aquaculture, or during processing (Amagliani et al., 2012). Our results indicated significant occurrence of *Salmonella* in both meat and seafood sold at local markets in Phnom Penh.

Moreover, analysis of fresh vegetables revealed *Salmonella* spp. prevalence of 33%. A previous study in Cambodia found that 57% and 15% of lettuce samples tested positive for *Salmonella* during the dry and rainy season, respectively (Desiree et al., 2021). In another study, *Salmonella* spp. was detected in 25% (78/310) of samples collected from vegetable food contact surfaces and non-food contact surfaces in markets in Cambodia (Schwan et al., 2021). We observed slightly higher occurrence of *Salmonella* on vegetables than reported in some neighboring countries, where *Salmonella* was detected in e.g., 23% (28/120) of samples of green leaf lettuce collected from open markets and supermarkets in Cambodia and Thailand (Chhay et al., 2018), in 13% (74/572) of retail fresh vegetables sampled in Vietnam (Nguyen et al., 2021), and in 33% (26/80) of various vegetables collected from retail markets in Laos PDR (Meunsene et al., 2022). While the occurrence of *Salmonella* in vegetable samples was lower than in meat and seafood samples, our results still indicated high levels of contamination with *Salmonella* among vegetables sold in the selected local markets and farms.

The *Salmonella* plate count data revealed no significant difference between meat, seafood, and vegetable samples, with the maximum count being just above 7.0 log₁₀ CFU/g (for seafood). The average counts were higher than those reported in a previous study in Cambodia, which detected 3–4 log₁₀ CFU/g in poultry samples (Lay et al., 2011), but similar to levels reported for on fresh lettuce collected from local farms and markets in Phnom Penh, Kampong Speu and Kandal province, Cambodia, with mean values ranging from 4.24 to 7.62 log₁₀ CFU/g (Chhim et al., 2022). This high contamination of fresh vegetables, meat, and seafood with *Salmonella* spp. indicates a considerable risk of infection on eating fresh food products. Therefore, an effective pretreatment process should be applied before consumption, and cross-contamination should be avoided.

Furthermore, the study also showed that serogroup C was the most prevalent *Salmonella* serogroup in fresh food samples collected from local markets and farms around Phnom Penh. A previous review showed that serogroup C is the most common serogroup in the United States, and its serovars are found increasingly in Europe and the United States, accounting for the majority of human infections (Fuche et al., 2016). Strains in serogroup C include Infantis, Thompson, Rissen, Newport, and Virchow, among others (Grimont and Weill, 2007; Herrera-León et al., 2007). *Salmonella enterica* serovar Rissen is reported to be the most common serotype (29% prevalence) in meat products in Cambodian border provinces, while *S. enterica* serovar Virchow has been found in 9.1% of broiler chickens in Egypt (Trongjit et al., 2017; Moawad et al., 2022). The second most common serogroup in our study was serogroup B, with serovars such as Typhimurium, Agona, Paratyphi B, Indiana, Haifa, Derby, and Stanley (Grimont and Weill, 2007; Herrera-León et al., 2007). Many serovars in this group are commonly found in food samples, e.g., studies of chickens, pigs, and meat products in the border provinces of Thailand have found that the most common serovar is *S. Typhimurium* (29%) (Trongjit et al., 2017; Gomes et al., 2022). These findings indicate that human salmonellosis can be linked with food sources at both farm and local market level.

The on-site observations described some general hygiene factors that could partly explain the high prevalence and counts of *Salmonella* in meat, seafood, and vegetables, such as varying temperature (ranging from 19 to 36 °C), improper storage and processing before transfer to the market, and long-distance transportation. At high temperatures, *Salmonella* will not only survive but also grow. It has been shown that *Salmonella* can not only survive, but actively grow at temperatures ranging from 2 to 54 °C depending on the serotype (Pui et al., 2011; Bintsis, 2017). Lack of an appropriate hygiene program is suggested to be the main factor behind the spread of *Salmonella* infection through freshwater fish (Bibi et al., 2015). Lack of sanitation and hygiene practices in vegetable production may also result in high levels of *Salmonella* survival and growth on vegetables sold in local markets (Desiree et al., 2020). In addition, Cambodian's poultry production largely relies on traditional or small-scale systems, which often lack effective practices and management. Despite the provision of food safety and hygiene training programs for producers and for government staff in the Cambodian livestock sector, many small-scale animal production systems show limited implementation of good food safety and hygiene practices (Birhanu et al., 2021). Moreover, a quantitative microbial risk assessment for salmonellosis among Cambodian consumers indicated an 11.1% probability of illness per person per year. This risk is potentially associated with cross-contamination during food preparation and raw material contamination at the market level (Rortana et al., 2022). These findings indicate that in combination, insufficient access to resources, inadequate training, limitations of the regulatory process, and the requirement for substantial shifts in behavior on both individual and community levels can lead to gaps in translating food safety knowledge into actual food safety practices. More attention to risk factors and a good understanding of *Salmonella* epidemiology in food products between farms and markets would minimize the negative impact on consumers. This study can provide essential data for future research design and influence plans emphasizing the necessity of a One-Health approach to safeguard public health from *Salmonella* in fresh food products from farms and markets.

5. Conclusions

Science-based data on the prevalence of foodborne pathogens and on existing food safety practices are needed as a basis for interventions to improve food safety and public health in low and middle-income countries. High prevalence and high levels of *Salmonella* in food products sold both at informal, and formal, markets can be the result of cross-contamination, as well as from the survival and growth of the bacteria at any point in the food-value chain. In the present study, a high prevalence

of *Salmonella* was confirmed and contributing risk factors were identified during production, transport, processing and sale of foods. To mitigate the adverse health impact of food borne bacteria, controlling or eliminating the spread of *Salmonella* spread in the food-value chain is essential. This can be achieved by enhancing the food safety practices of farmers, retailers, and consumers.

CRedit authorship contribution statement

Laingshun Huoy: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sireyvathanak Vuth:** Methodology, Investigation. **Sophanith Hoeng:** Methodology, Investigation. **Chilean Chheang:** Methodology, Investigation. **Phalla Yi:** Methodology, Investigation. **Chenda San:** Methodology, Investigation. **Panha Chhim:** Methodology, Investigation. **Sopacpear Thorn:** Methodology, Investigation. **Bunsopheana Ouch:** Methodology, Investigation. **Den-grachda Put:** Methodology, Investigation. **Lyna Aong:** Methodology, Investigation. **Kongkea Phan:** Writing – review & editing, Supervision, Investigation. **Leila Nasirzadeh:** Writing – review & editing, Supervision, Conceptualization. **Siteng Tieng:** Writing – review & editing, Supervision, Conceptualization. **Erik Bongcam-Rudloff:** Writing – review & editing, Supervision, Conceptualization. **Susanna Sternberg-Lewerin:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Sofia Boqvist:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Formal analysis, Data curation, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fm.2024.104614>.

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Antimicrobial resistance and serotype distribution of *Salmonella* spp. isolated from fresh foods in Cambodia

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Abstract

Aims: To determine the *Salmonella* serotype distribution, antimicrobial resistance profiles, and antimicrobial resistance genes (ARGs) in food samples obtained from local markets in a low-income urban setting and nearby farms in Cambodia.

Methods and results: One hundred and thirty-nine *Salmonella* isolates from various food sources were tested for antibiotic susceptibility using a panel of 12 antibiotics, and 81 selected *Salmonella* isolates were further sequenced for serotype distribution and ARG identification. The results showed that 71% (99/139) of the isolates exhibited resistance to at least one antibiotic, with 39% (39/99) classified as multidrug-resistant (MDR). The highest resistance was observed against azithromycin (37%), followed by oxytetracycline (35%). A total of 32 serotypes were identified, with the six most common being *S. Corvallis* (7%), *S. Haifa* (6%), *S. Weltevreden* (6%), *S. Agona* (5%), *S. Kentucky* (5%), and *S. Livingstone* (5%). A broad range of ARGs was observed across multiple antibiotic classes, including macrolides, aminoglycosides, tetracyclines, phenicols, fluoroquinolones, sulfonamide–trimethoprim, beta-lactams, and MDR genes.

Conclusions: The results highlight the potential role of fresh food products in the widespread dissemination of *Salmonella* strains resistant to multiple antibiotics.

Impact Statement

This study demonstrates the need for targeted food safety measures and antimicrobial stewardship, particularly in low- and middle-income countries.

Keywords: *Salmonella* serotype; multidrug resistance; antimicrobial resistance genes; food safety

Introduction

Food safety aims to ensure the availability of safe, high-quality food products for consumers worldwide. The safety level is related to foods free from contaminants, including foodborne pathogens such as bacteria and other harmful microorganisms, chemical pollutants such as heavy metals, pesticides, and pharmaceutical residues, physical contaminants, and allergens (Wu et al. 2021, Thakali et al. 2022, Tibebe et al. 2024). Addressing public health concerns and international food trade requires collaboration between consumers, governments, international organizations, and industries to ensure food safety through adequate regulations, guidelines, and access to appropriate resources (WHO 2022). Food safety regulations must acknowledge the food safety link between food production and consumption at all levels within the food system. Research on food safety interventions implemented at the market level in low- and middle-income countries (LMICs) highlights the

effectiveness gap of such measures for both vendors and consumers (Kwoba et al. 2023). This gap arises from insufficient regulation of microbial contamination and lack of implementation of regulations, which ultimately results in inadequate food safety management within the food production chain in many LMICs, including Cambodia.

In Cambodia, food safety remains a significant concern for public health, economic improvement, and the promotion of sustainable agriculture development (Mosimann et al. 2023). *Salmonella* has been described as one of the most commonly found foodborne agents in multitudinous fresh food products in LMICs, including Cambodia (Lettini et al. 2016, Trongjit et al. 2017, Desiree et al. 2021, Patra et al. 2021, Nguyen et al. 2021c). More than 2600 serotypes have been recognized within the *S. enterica* species (Ferrari et al. 2019). The most frequently reported serotypes among European countries include *S. Typhimurium*, *S. Kentucky*, and *S. Enteritidis* (EFSA 2024). In Asia, *S. Typhimurium* has been identified as the pre-

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Table 1. *Salmonella enterica* isolated from various food commodities from food markets and vegetable farms in Phnom Penh, Cambodia (Huoy et al. 2024).

Samples source	Number of isolates from								Morning glory	Curly cabbage	Total ^a
	Pork	Beef	Chicken	Fish	Seafood	Bok choy	White cabbage	Salad			
Markets	18	18	17	16	16	8	3	12	11	9	128
Farms						2			8	1	11
Total	18	18	17	16	16	10	3	12	19	10	139

^aThese isolates were last confirmed with a PCR screening test using the *invA* gene.

dominant serotype among non-typhoidal strains, while *S. Typhi* was recognized as the main serotype within the typhoidal strains (Patra et al. 2021, Salvador et al. 2022, Wang et al. 2023b). Regarding Cambodia, several serotypes have been reported from meat products and food contact surfaces, including *S. Typhimurium*, *S. Rissen*, *S. Hvittingfoss*, *S. Corvallis*, *S. Krefeld*, *S. Weltevreden*, *S. Altona*, and *S. Anatum* (Lay et al. 2011, Trongjit et al. 2017, Schwan et al. 2021). In addition, *S. Typhi*, *S. Paratyphi A*, and *S. Choleraesuis* were documented as the cause of typical clinical *Salmonella* infection among hospitalized adults in Cambodia (Vlieghe et al. 2012, Kuijpers et al. 2017, Kheng et al. 2020). Indeed, Kheng et al. (2020) reported that *S. Typhi* was the primary serovar in clinical salmonellosis in 2012–2013 (94% of cases), while *S. Paratyphi A* accounted for 61% of infections in 2014.

Antimicrobials are often used to treat and control the spread of *Salmonella* spp. and other bacterial infections among humans, livestock, and crops/horticulture (Crump et al. 2015, Givens et al. 2023). Antibiotics are also used for prophylactic purposes and as growth promoters in the livestock industry (Peng et al. 2014, Van Boeckel et al. 2019, Van et al. 2020). The most common antimicrobial classes used to treat clinical *Salmonella* infection in humans are carbapenems, penicillins, fluorquinolones, and cephalosporins (WHO 2019, Nambiar et al. 2024). However, the rise in antimicrobial resistance (AMR) presents a considerable threat to public health, and prevention, as opposed to treating bacterial infections including salmonellosis, is becoming increasingly critical (Vlieghe et al. 2013, Trongjit et al. 2017).

The development of AMR is accelerated through the lack of control over antibiotic usage and limited knowledge regarding the application of antibiotics in livestock farms, notably in LMICs (Heyman 2020, Mann et al. 2021). Globally, tetracycline, penicillins, and macrolides are commonly used in agriculture and livestock production (Laxminarayan et al. 2015, Mann et al. 2021). However, in Cambodia, the sale of antibiotics is poorly regulated, which leads to antibiotic purchases without a prescription (Reed et al. 2019, Lim et al. 2021). This, together with limited knowledge of proper antibiotic usage, generates an increased risk of the development and spread of AMR (Om and McLaws 2016, Chea et al. 2022). Multidrug resistance (MDR), resistance to at least three classes of antimicrobials (Lettini et al. 2016, Catalano et al. 2022), is of particular concern in Cambodia, with reports showing that 52% of the *Salmonella* isolates collected from pigs and broiler chickens from local markets were multidrug resistant (Trongjit et al. 2017). Another study showed that ~88% of *S. Typhi* isolated from Cambodian children between 2012 and 2016 exhibited MDR (Kheng et al. 2020). Moreover, studies have shown that most *S. Typhi* and *S. Paratyphi* isolates were resistant to ciprofloxacin (Kuijpers et al. 2017, Gandra et al.

2020). Thus, one can conclude that increasing AMR among *Salmonella* strains in Cambodia poses severe challenges to food safety and public health.

Resistant bacteria and antimicrobial resistance genes (ARGs) transmit through the food chain, and this is particularly challenging within LMICs. There are several reasons for the transmission routes from primary producer to food retailer, for example, lack of surveillance, poor biosecurity, and informal production chains (Sagar et al. 2023). There is also a lack of data on circulating *Salmonella* serotypes and phenotypic and genotypic AMR in the food production system in Cambodia. Such data are essential when developing strategies and interventions to address food safety challenges at various levels in the food system. This study aimed to determine the *Salmonella* serotype distribution, AMR profiles, and ARGs of *Salmonella* isolates from fresh food samples collected from local markets in the capital region of Cambodia and in vegetable farms supplying the urban markets.

Materials and methods

Salmonella isolates

Between 2020 and 2021, a study was performed at food markets in the Cambodian capital Phnom Penh and at vegetable farms adjacent to Phnom Penh to investigate the prevalence of *Salmonella* among three categories of fresh food: meat, seafood, and vegetables (Huoy et al. 2024). A total of 139 isolates from 285 food samples from that study were used in this study (Table 1). The sampling process, *Salmonella* cultivation, and confirmation are described in detail by Huoy et al. (2024).

Antimicrobial susceptibility tests

Antimicrobial susceptibility tests (ASTs) were performed with the Kirby–Bauer disk diffusion method for the 12 included antibiotics: azithromycin (Azm), cefuroxime (Cxm), doxycycline (DO), ampicillin (Amp), imipenem (Ipm), sulfamethoxazole-trimethoprim (SxT), aztreonam (Atm), ciprofloxacin (Cip), chloramphenicol (C), oxytetracycline (OT), gentamicin (Gn), and amoxicillin (Aml) (Table 2). All 139 frozen (–20°C) isolates were thawed and enriched in nutrient broth (NB, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), followed by sub-culturing on *Salmonella-Shigella* agar (SS, HiMedia Laboratories Private Limited, Maharashtra, India). Five isolated colonies per plate were inoculated in NB (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and incubated at 37°C in a shaking incubator for 6 h. The bacterial culture was adjusted to 0.5 McFarland turbidity standards and spread onto Mueller–Hinton agar (HiMedia Laboratories Private Limited, Maharashtra, India). Antibiotic discs were applied to the plates, which were incubated for 24 h at 37°C. The zone of inhibition was measured and interpreted according to

Table 2. The standard zone size of antimicrobial disks used in a study investigating *Salmonella* spp. among various food samples in Cambodia (CLSI 2020).

Antibiotic class	Antibiotic substance	Disk content (ug)	Zone diameter breakpoints, nearest whole mm		
			Susceptible (S)	Intermediate (I)	Resistance (R)
Penicillin ^a	Ampicillin (Amp)	10	≥17	14–16	≤13
	Amoxicillin (Aml)	25	≥18	14–17 ^b	≤13
Cephem (cephalosporin) ^a	Cefuroxime (Cxm)	30	≥18	15–17 ^b	≤14
Monobactams ^a	Aztreonam (Atm)	30	≥21	18–20 ^b	≤17
Carbapenems ^a	Imipenem (Ipm)	10	≥23	20–22 ^b	≤19
Aminoglycosides	Gentamycin (Gn)	10	≥15	13–14 ^b	≤12
Macrolides	Azithromycin (Azm)	15	≥13		≤12
Tetracycline	Doxycycline (Do)	30	≥14	11–13	≤10
	Oxytetracycline (Ot)	30	>15	12–14	<11
Quinolones	Ciprofloxacin (Cip)	5	≥31	21–30 ^b	≤20
Folate pathway antagonists	Sulfonamide–trimethoprim (Sxt)	25	≥16	11–15	≤10
Phenicol	Chloramphenicol (C)	30	≥18	13–17	≤12

^aMonobactam; carbapenems; cephalosporin; and penicillin are subclasses of beta-lactam antibiotics.

^bIntermediate breakpoints for corresponding antibiotic substance that can potentially concentrate at an anatomical site.

the Clinical Laboratory Standard Institute (CLSI) (Table 2) (CLSI 2020). Resistance to at least three classes of antibiotics was defined as MDR. *Escherichia coli* ATCC 25922 with *S. enterica* subspecies *enterica* serotype Typhimurium ATCC 14028 was used as a control strain.

Salmonella whole genome sequencing

Initially, we planned to sequence all 139 isolates. However, certain of the DNA samples transported to Sweden failed to meet the quality requirements for sequencing. Therefore, isolate selection was prioritized for isolates containing high-quality DNA and having at least one antibiotic resistance, as our goal was to compare their AMR profiles with predicted resistance genes (ARGs) from sequencing data. Additionally, three of these strains were selected as references, meaning they displayed no resistance.

In total, 81 out of 139 *Salmonella* spp. isolates were selected for whole genome sequencing (WGS) to determine their serotypes and ARGs. Briefly, *Salmonella* genomic DNA was extracted using the Wizard® HMW DNA extraction kit (Promega, Madison, USA). DNA quality check was performed using a NanoDrop™ 8000 Spectrophotometer (Thermo Fisher Scientific, Delaware, USA) and a Qubit 4.0 Fluorometer (Thermo Fisher Q33238, Invitrogen, USA). Samples with OD_{260/280} = 1.8–2.0 and a minimum concentration of 2.5 µg were selected for sequencing library preparation using TruSeq PCR-free DNA library preparation kit (Illumina Inc.). Sequencing was conducted using NovaSeq X 10B lane with paired-end sequencing of 150 cycles (Illumina, SciLife Lab, Uppsala, Sweden).

Salmonella whole genome sequence analysis

Sequence quality control and trimming

FastQC (Andrews 2010) was used to assess the quality of the raw Illumina sequencing reads. Read quality was improved using Trimmomatic (Bolger et al. 2014), which removed adapter sequences and filtered out low-quality reads (Phred score < 25) with default parameters.

Serotype prediction

Serotype prediction was performed on quality-controlled sequencing reads using SeqSero2 (Zhang et al. 2019), which utilized a reference database for *Salmonella* serotyping.

Whole-genome assembly

Genome assemblies were generated from quality-controlled Illumina short reads using SPAdes v3.15.5 with the careful parameter to reduce mismatches and short indels. The default *k*-mer sizes (21, 33, 55, and 77) were used. Assembly quality was evaluated using QUAST v5.0.2 based on total assembly size, N50, and the number of contigs. Assemblies with N50 > 30 kb and fewer than 500 contigs were considered suitable for downstream ARG prediction using AMR tools.

Antibiotic resistance gene identification

Predictive identification of ARGs was conducted using the Comprehensive Antibiotic Resistance Database (CARD) Resistance Gene Identifier (RGI) tool. Genome assemblies were used as input for the CARD-RGI tool.

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

Results

AMR of the *Salmonella* isolates

Among the 139 *Salmonella* isolates, 99 (71%) exhibited resistance to at least one antibiotic, with 39 (39%) of these identified as MDR. The highest proportion of resistant isolates was observed against azithromycin (37%), followed by oxytetracycline (35%), ampicillin (24%), amoxicillin (24%), doxycycline (20%), chloramphenicol (18%), sulfamethoxazole–trimethoprim (17%), cefuroxime (14%), gentamicin (12%), ciprofloxacin (8%), aztreonam (8%), and imipenem (4%) (Table 3).

There was intermediate resistance (I) against ciprofloxacin in 41% (57/139) of the included samples and against gentamicin in 30% (42/139) (Fig. 1). The highest proportions of sus-

Table 3. Antimicrobial resistance in 139 *Salmonella* spp. isolated from meat, seafood, and vegetables in Cambodia.

Sample type	Number of isolates	Antimicrobial resistance (% resistant isolates) against tested antimicrobial agents											
		Azm	Cxm	Do	Amp	Ipm	Sxt	Atm	Cip	C	Ot	Gn	Aml
Meat	53	8	21	25	28	4	23	11	4	26	45	6	26
Seafood/fish	32	47	16	22	19	9	16	3	19	9	22	9	19
Vegetable	54	59	7	15	24	0	13	7	6	15	31	20	24
Total	139	37	14	20	24	4	17	8	8	18	35	12	24

Azm = azithromycin, Cxm = cefuroxime, Do = doxycycline, Amp = ampicillin, Ipm = imipenem, Sxt = sulfamethoxazole–trimethoprim, Atm = aztreonam, Cip = ciprofloxacin, C = chloramphenicol, Ot = oxytetracycline, Gn = gentamicin, Aml = amoxicillin.

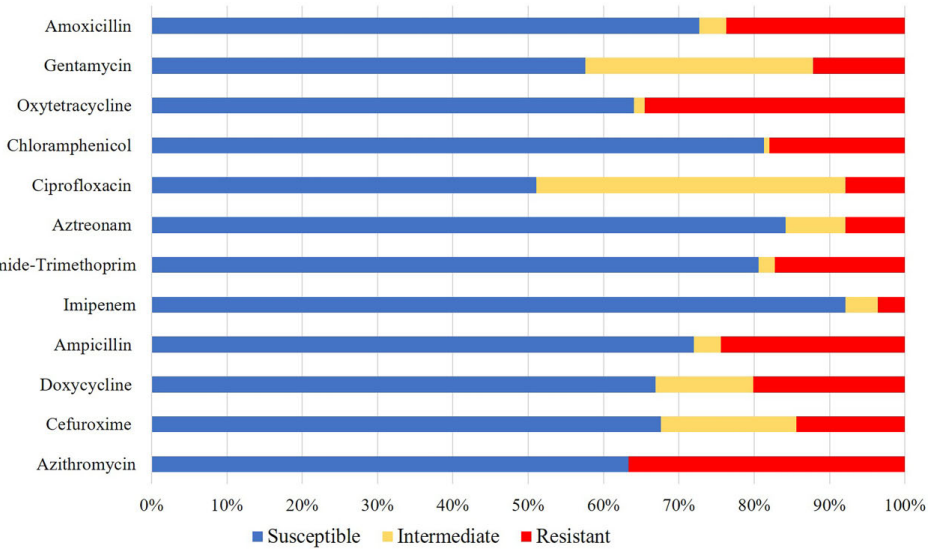


Figure 1. Percentage (%) of antimicrobial susceptibility exhibited among *Salmonella* spp. ($n = 139$), isolated from different food commodities collected in Cambodia, for each of the 12 included antibiotics.

ceptible isolates were observed for imipenem, aztreonam, and chloramphenicol.

***Salmonella* serotype distribution and AMR phenotypes**

A total of 81 *Salmonella* spp. isolates were submitted for Illumina sequencing. Among these, 75 isolates were classified into 32 distinct serotypes belonging to serogroups B ($n = 17$), C ($n = 36$), E ($n = 13$), F ($n = 1$), G ($n = 2$), I ($n = 4$), and R ($n = 2$) (Table 4). A greater diversity of serotypes was identified among the isolates from vegetables compared to the other food sources. Additionally, six isolates could not be assigned to specific serotypes. Among these, one isolate belonged to serogroup D, another to serogroup I, while four isolates could not be classified into any serogroup.

The most common serotypes were *S. Corvallis* (7%), *S. Haifa* (6%), *S. Weltevreden* (6%), *S. Agona* (5%), *S. Kentucky* (5%), *S. Livingstone* (5%), *S. Typhimurium* (4%), *S. Infantis* (4%), *S. Rissen* (4%), *S. Bareilly* (4%), *S. Mbandaka* (4%), *S. Uganda* (4%), and *S. Hvittingfoss* (4%) (Table 4).

The phenotypic AMR profiles were categorized based on the number of antimicrobial classes to which the strain exhibited resistance, ranging from at least 1 to 9 classes (Table 5). Approximately 41% (31 out of 75) identified *Salmonella* serotypes exhibited the MDR phenotype. Among the identified resistance profiles, significant MDR was observed in four *Salmonella* isolates. This included two isolates from vegetable sources: one *S. Weltevreden*, which was resistant to nine antibiotic classes and one *S. Corvallis*, which was resistant to eight antibiotic classes. Furthermore, three isolates displayed resistance to seven or eight antibiotic classes.

Distribution of ARGs

A total of 144 ARGs were detected among the 81 *Salmonella* genomes (Table 6). The identified ARGs were associated with various antimicrobial classes examined in this study, such as beta-lactams (including monobactam, carbapenems, cephalosporin, and penicillin), tetracyclines, aminoglycosides, quinolones, phenols, sulfonamide–trimethoprim, and macrolides. Resistance to other antibiotic categories was also predicted from this database, including cephamycins, gly-

Table 4. Serotype distribution of 81 *Salmonella* spp. isolated from different food commodities in Cambodia.

Serogroup	Serotypes	Antigenic formulae	Number of isolates (%)				Total
			Pork/beef	Poultry meat	Seafood/fish	Vegetable	
B	S. Haifa	4:z10:1,2	1 (1.2)	1 (1.2)	1 (1.2)	2 (2.5)	5 (6.2)
	S. Agona	4:f,g,s:-	3 (3.7)	1 (1.2)	-	-	4 (5.0)
	S. Typhimurium	4:i:1,2	-	-	1 (1.2)	2 (2.5)	3 (3.7)
	S. Indiana	4:z:1,7	-	-	-	1 (1.2)	1 (1.2)
	S. Heidelberg	4:r:1,2	1 (1.2)	-	-	-	1 (1.2)
	S. Saintpaul	4:e,h:1,2	-	-	1 (1.2)	-	1 (1.2)
	S. Chester	4:3,h:e,n,x	-	-	1 (1.2)	-	1 (1.2)
	S. Brancaster	4:z29:-	-	1 (1.2)	-	-	1 (1.2)
C	S. Corvallis	8 :z4,z23:-	1 (1.2)	3 (3.7)	-	2 (2.5)	6 (7.4)
	S. Kentucky	8:i:z6	1 (1.2)	1 (1.2)	1 (1.2)	1 (1.2)	4 (5.0)
	S. Livingstone	7:d:l,w	2 (2.5)	-	2 (2.5)	-	4 (5.0)
	S. infantis	7 : r :1,5	-	2 (2.5)	1 (1.2)	-	3 (3.7)
	S. Rissen	7:f,g:-	2 (2.5)	-	-	1 (1.2)	3 (3.7)
	S. Bareilly	7:y:1,5	1 (1.2)	-	1 (1.2)	1 (1.2)	3 (3.7)
	S. Mbandaka	7:z10:e,n,z15	-	1 (1.2)	-	2 (2.5)	3 (3.7)
	S. Thompson	7:k:1,5	-	-	-	2 (2.5)	2 (2.5)
	S. Braenderup	7:e,h:e,n,z15	-	-	2 (2.5)	-	2 (2.5)
	S. Molade/S. Wippra	8:z10:z6	-	-	1 (1.2)	1 (1.2)	2 (2.5)
	S. Newport	8:e,h:1,2	-	-	-	1 (1.2)	1 (1.2)
	S. Mkamba	7:l,v:1,6	-	-	1 (1.2)	-	1 (1.2)
	S. Potsdam	7:l,v:e,n,z15	-	-	-	1 (1.2)	1 (1.2)
	S. Tananarive/S. Brunei	8:y:1,5	-	-	-	1 (1.2)	1 (1.2)
	Other strain ^a	9,46:r:-	-	-	-	1 (1.2)	1 (1.2)
E	S. Weltevreden	3,10:r:z6	-	-	1 (1.2)	4 (5.0)	5 (6.2)
	S. Uganda	3,10:l,z13:1,5	2 (2.5)	-	-	1 (1.2)	3 (3.7)
	S. Anatum	3,10:e,h:1,6	2 (2.5)	-	-	-	2 (2.5)
	S. London	3,10:l,v:1,6	2 (2.5)	-	-	-	2 (2.5)
	S. Give	3,10:l,v:1,7	1 (1.2)	-	-	-	1 (1.2)
F	S. Aberdeen	11:i:1,2	1 (1.2)	-	-	-	1 (1.2)
G	S. Kedougou	13:i:l,w	-	-	1 (1.2)	1 (1.2)	2 (2.5)
I	S. Hittingfoss	16:b:e,n,x	-	1 (1.2)	-	2 (2.6)	3 (3.7)
	S. Wa	16:b:1,5	-	-	1 (1.2)	-	1 (1.2)
	Other strain ^b	16:r:e,n,x	-	-	-	1 (1.2)	1 (1.2)
R	S. Johannesburg	40:b:e,n,x	2 (2.5)	-	-	-	2 (2.5)
Others	Other subspecies I	67:-:z6	-	1 (1.2)	-	-	1 (1.2)
	Unidentified strains	-	1 (1.2)	-	1 (1.2)	1 (1.2)	3 (3.7)
Total			23 (28)	12 (15)	17 (21)	29 (36)	81 (100)

^aThe antigenic formula is possibly closely related to the strains S. Deckstein (9,46: r:1,7)/S. Shoreditch (9,46: r: e, n, z15)/S. Sokode (9,46: r: z6).

^bThe antigenic formula is possibly closely related to the strain S. Annedal (16: r: i: e, n, x).

copeptides, lincosamides, nucleosides, peptides, phosphonic acid, pleuromutins, rifamycins, and agents used for disinfection and antiseptics. Additionally, MDR genes, i.e. ARGs encoding resistance mechanisms against several different antibiotics, such as efflux pumps, were detected among the isolates, with the commonly identified genes being *sdiA*, *marA*, *acrB*, *rsmA*, *golS*, *mdsA*, *mdsB*, and *mdsC*, among others. Furthermore, genes associated with resistance to disinfectants and antiseptics, such as *qacG*, *qacL*, and *qacEdelta1*, were also identified. Six resistance mechanisms were observed among the sequence data, including antibiotic efflux, antibiotic inactivation, target alteration, target replacement, target protection, and reduced permeability to the antibiotic substance.

AMR phenotype and genotype matching

Table 7 presents the matching percentage between the AMR phenotype and genotype of the studied isolates. Agreement between AMR pattern and the corresponding resistance genes

was noted across several antimicrobial classes, including macrolides, folate pathway antagonists, quinolones, phenicols, tetracyclines, and aminoglycosides. Additionally, a clear association between MDR genes (*golS*, *mdsA*, *mdsB*, and *mdsC*) and phenotypic resistance to antimicrobial classes such as cephalosporins, carbapenems, and monobactams was observed. In contrast, a low matching percentage was seen between phenotype and genotype for amoxicillin resistance, with only a 24% match.

Discussion

Salmonella is a major contributor to foodborne illnesses globally. Numerous Cambodian studies have reported a high prevalence of *Salmonella*-contaminated food and strains that have been shown to exhibit high levels of AMR (Kheng et al. 2020, Trongjit et al. 2017). Consequently, it is crucial to understand the distribution of *Salmonella* serotypes, the profiles of AMR, and the mechanisms driving resistance by identify-

Table 5. Antimicrobial resistance phenotypic identified among the *Salmonella* serovars isolated from various foods in Cambodia.

Serovars (Number of resistant isolates/total number of isolates)	Number of isolates	Resistance profile ^a	Number of antimicrobial classes ^b
S. Corvallis (2/6), S. Typhimurium (2/3), S. Newport (1/1), S. Livingstone (2/4), S. Kentucky (1/4), S. Bareilly (1/3), S. Mbandaka (1/3), S. Kedougou (1/2), S. Braenderup (1/2), S. Thompson (1/2), S. Weltevreden (1/5), serotype 16:r:e,n,x (1/6)	15	Azm	1
S. Corvallis (1/6), S. Bareilly (1/3)	2	Atm	1
S. Johannesburg (2/2), S. Chester (1/1), S. Give (1/1)	4	Cxm	1
S. Anatum (1/2), S. London (1/2)	2	Ot	1
S. Anatum (1/2), S. Haifa (1/5)	2	Do	1
S. Kentucky (1/4)	1	Sxt	1
S. Molade/S. Wippra (1/2), S. Haifa (1/5)	2	Do-Ot	1
Serotype 9,46:r:- (1/1)	1	Amp-Aml	1
S. Agona (1/4), S. Heidelberg (1/1), S. Haifa (1/5)	3	C-Ot	2
S. Bareilly (1/3), S. Mbandaka (1/3)	2	Azm-Gn	2
S. Hvitittingfoss (1/3)	1	Azm-Ot	2
S. Livingstone (1/4)	1	Azm-Cip	2
S. Livingstone (1/4)	1	Cxm-Gn	2
S. Corvallis (1/6)	1	Ipm-Ot	2
S. Molade/S. Wippra (1/2)	1	Azm-Ipm	2
S. Haifa (1/5), S. Corvallis (1/6)	2	Azm-Do-Ot	2
S. Mbandaka (1/3)	1	Do-C-Ot	2
Serotype 67:-:Z6 (1/1)	1	Amp-Sxt-Aml	2
S. Hvitittingfoss (1/3)	1	Cxm-Amp-Aml	2
S. Thompson (1/2)	1	Azm-Amp-Gn	3
S. Livingstone (1/4)	1	Azm-Atm-Cip	3
S. Weltevreden (1/5)	1	Azm-Sxt-Ot	3
S. Weltevreden (1/5)	1	Azm-Do-Ipm-Ot	3
S. Haifa (1/5)	1	Azm-Do-Cip-Ot	3
S. Tananarive/S. Brunei (1/1)	1	Azm-Amp-Ot-Aml	3
S. Typhimurium (1/3)	1	Azm-Amp-C-Aml	3
S. Uganda (1/3)	1	Azm-Amp-Gn-Aml	3
S. Wa (1/1)	1	Azm-Cxm-Amp-Aml	3
S. Agona (1/4)	1	Cxm-Amp-Ot-Aml	3
S. Kentucky (1/4)	1	Amp-Cip-Ot-Aml	3
S. Haifa (1/5)	1	Amp-Sxt-Ot-Aml	3
S. Kedougou (1/2)	1	Do-Amp-C-Aml	3
S. Rissen (1/3), S. Agona (1/4), S. London (1/2)	3	Amp-Sxt-C-Ot-Aml	4
S. Brancaster (1/1)	1	Do-Amp-Sxt-C-Ot-Aml	4
S. Braenderup (1/2)	1	Cxm-Do-Amp-Cip-Ot-Aml	4
S. infantis (1/3)	1	Cxm-Do-Amp-C-Ot-Aml	4
S. Agona (1/4)	1	Cxm-Do-Amp-Sxt-C	5
S. Indiana (1/1)	1	Cxm-Amp-Sxt-Atm-Ot-Aml	5
S. infantis (1/3)	1	Cxm-Do-Sxt-Atm-C-Ot-Gn	6
S. Potsdam (1/1), S. Rissen (1/3)	2	Azm-Amp-Sxt-C-Ot-Gn-Aml	6
S. Mkamba (1/1)	1	Amp-Sxt-Cip-C-Ot-Gn-Aml	6
S. Saintpaul (1/1)	1	Azm-Do-Amp-Sxt-Cip-C-Ot-Aml	6
S. Uganda (1/3)	1	Cxm-Do-Amp-Atm-C-Ot-Gn-Aml	6
S. Kentucky (1/4)	1	Azm-Cxm-Do-Amp-Sxt-Cip-C-Ot-Aml	7
S. Infantis (1/3)	1	Cxm-Do-Amp-Sxt-Atm-Cip-Ot-Gn-Aml	7
S. Corvallis (1/6)	1	Cxm-Do-Amp-Sxt-Atm-Cip-C-Ot-Gn-Aml	8
S. Weltevreden (1/5)	1	Azm-Cxm-Do-Amp-Sxt-Atm-Cip-C-Ot-Gn-Aml	9
Total number of isolates	75		

^aAMR abbreviations: Azm = azithromycin, Cxm = cefuroxime, Do = doxycycline, Amp = ampicillin, Ipm = imipenem, Sxt = sulfamethoxazole-trimethoprim, Atm = aztreonam, Cip = ciprofloxacin, C = chloramphenicol, Ot = oxytetracycline, Gn = gentamicin, Aml = amoxicillin.

^bAntimicrobial classes: macrolide (Azm), cephalosporing (Cxm), tetracycline (Do, Ot), penicillin (Amp, Aml), carbapenems (Ipm), sulfonamide/trimethoprim (Sxt), monobactams (Atm), quinolones (Cip), phenicol (C), aminoglycosides (Gn).

ing ARGs. In the current study, 139 *Salmonella* isolates collected from different food commodities (meat, seafood/fish, and vegetables) described in a previous study (Huoy et al. 2024) were serotyped and characterized for phenotypic and genotypic AMR.

Analysis of the 139 *Salmonella* isolates revealed a high prevalence of resistance to azithromycin and oxytetracycline, with the second-highest resistance observed in two widely

used penicillin-class antibiotics, ampicillin and amoxicillin. These findings are consistent with several studies conducted in Cambodia, other Southeast Asian countries, and various European Union (EU) member states. Over a 10-year period, studies on AMR indicated a rising resistance rate of 53%–77% among *Salmonella* isolates from human, animal, and environment samples in South Asia, with particularly high resistance to tetracycline and amoxicillin (Talukder et

Table 6. ARG detection by sequence analysis using CARD-RGI on *Salmonella* isolates from food samples collected in Cambodia.

Antimicrobial classes	Antimicrobial resistance genes (ARGs)
Beta-lactam	ACC-1a, TEM-1, TEM-176, TEM-215, CMY-159, CMH-3, CTX-M-55, CTX-M-65, LAP-2, OXA-1, OXA-10, Sed-1, SHV-11, SHV-26, LptD
Tetracycline	<i>tet(A)</i> , <i>tet(B)</i> , <i>tet(I)</i> , <i>tet(L)</i> , <i>tet(M)</i> , <i>tet(45)</i> , <i>tet(X4)</i> , <i>tetR</i> , <i>emrK</i>
Aminoglycoside	AAC(3)-IIId, AAC(3)-IIe, AAC(3)-IVa, AAC(6')-Iaa, AAC(6')-Ib10, AAC(6')-If, AAC(6')-Ii, AAC(6')-Iid, AAC(6')-Iy, <i>aadA</i> , <i>aadA2</i> , <i>aadA3</i> , <i>aadA7</i> , <i>aadA16</i> , <i>aadA23</i> , <i>acrD</i> , APH(3')-Ia, APH(3')-Ib, APH(4)-Ia, APH(6)-Id, <i>baeR</i> , <i>baeS</i> , <i>cpxA</i> , <i>kdpE</i> , <i>mdtA</i> , <i>mdtB</i> , <i>mdtC</i>
Quinolone/fluoroquinolone	<i>emrA</i> , <i>emrB</i> , <i>emrR</i> , <i>MdtK</i> , <i>QepA2</i> , <i>QnrB12</i> , <i>QnrB19</i> , <i>QnrS1</i> , <i>QnrS2</i> , <i>QnrD1</i> , <i>gyrA</i> , <i>gyrB</i> , <i>parC</i> , <i>adeF</i>
Phenicol	<i>floR</i> , <i>catA4</i> , <i>catB3</i> , <i>cmlA1</i> , <i>cmlA5</i> , <i>catII</i> from <i>E. coli</i> K-12
Sulfonamide–trimethoprim	<i>sul1</i> , <i>sul2</i> , <i>sul3</i> , <i>dfrE</i> , <i>dfrA1</i> , <i>dfrA12</i> , <i>dfrA14</i>
Macrolide	<i>mphA</i> , <i>mef</i> (B), <i>Mrx</i> , <i>E. coli</i> <i>emrE</i> , <i>efmA</i> , CR P
MDR genes	<i>sdiA</i> , <i>marA</i> , <i>rsmA</i> , <i>ramA</i> , <i>mdtM</i> , <i>ogxB</i> , <i>ogxB</i> , <i>acr B</i> , <i>Ac r E</i> , <i>Acr F</i> , <i>AcrS</i> , <i>fosA5</i> , <i>acrA</i> , <i>AcrAB-TolC</i> with <i>A c r R mu t at ion</i> , <i>AcrAB- T olC</i> with <i>MarR mutation s</i> , <i>E. coli</i> <i>soxS mutation</i> , <i>E. coli</i> <i>sox R mu t at ion</i> , <i>K. pneumoniae</i> <i>acrR mu tati on</i> , <i>CRP</i> , <i>efrA</i> , <i>ErmB</i> , <i>evgA</i> , <i>gadW</i> , <i>H-NS</i> , <i>mdtE</i> , <i>msrC</i> , <i>KpnE</i> , <i>KpnF</i> , <i>KpnG</i> , <i>Kp nH</i> , <i>Md rQ</i> , <i>gols</i> , <i>mdsA</i> , <i>mdsB</i> , <i>mdsC</i> , <i>K. pneumoniae</i> <i>OmpK37</i> , <i>E. coli</i> <i>mdfA</i>
Disinfecting agents and antiseptics	<i>qacG</i> , <i>qacL</i> , <i>qacEdelta1</i>
Other ARGs	<i>ArnT</i> , <i>bacA</i> , <i>eptB</i> , <i>FosA2</i> , <i>FosA6</i> , <i>FosA7</i> , <i>FosA8</i> , <i>mdtG</i> , <i>OmpA</i> , <i>PmrF</i> , <i>ugd</i> , <i>MCR-1.1</i> , <i>E. coli</i> <i>GlpT mutation</i> , <i>E. coli</i> <i>UhpT mutation</i> , <i>msbA</i> , <i>eatAv</i> , <i>vanG</i> , <i>vanY</i> gene in (<i>vanA</i> , <i>vanB</i> , <i>vanF</i> , <i>vanM</i>) cluster, <i>vanT</i> gene in <i>vanG</i> cluster, <i>vanXY</i> gene in <i>vanC</i> cluster, <i>ln uA</i> , <i>lsaA</i>

Table 7. Matching percentage between phenotypic and genotypic antimicrobial resistance in *Salmonella* isolates isolated from various foods in Cambodia.

Antimicrobial class	Antimicrobial sub-class	Antimicrobial agent	Number of phenotypic resistance isolates	Number of isolates carrying antimicrobial resistance genes (ARGs)	Matching* AMR-ARGs (%)
Beta-lactam	Monobactams	Aztreonam (Atm)	9	9 ^a	100 00
		Cefuroxime (Cxm)	18	18 ^a	100 00
	Cephem (cephalosporin)	Ampicillin (Amp)	31	31 ^b	100 00
		Amoxicillin (Aml)	29	7	2414
	Carbapenems	imipenem (Ipm)	4	3 ^a	7500
Folate pathway antagonists	-	Sulfonamide–trimethoprim (Sxt)	21	21	100 00
Macrolides	-	Azithromycin (Azm)	36	36	100 00
Quinolones	-	Ciprofloxacin (Cip)	11	11	100 00
Phenicol	-	Chloramphenicol (C)	22	22	100 00
Tetracycline	-	Oxytetracycline (Ot)	38	38	100 00
	-	Doxycycline (Do)	23	23	100 00
Aminoglycosides	-	Gentamycin (Gn)	13	13	100 00

*% matching of AMR phenotype and genotype was calculated by dividing the total number of AMR phenotypic by the total number of isolates carrying ARGs.

^aMDR genes (*gols*, *mdsA*, *mdsB*, and *mdsC*) presented and responsible for the resistant mechanism to antibiotic classes (monobactam; carbapenem; cephalosporin; cephamycin; penam; phenicol antibiotic; and penem).

^bResistance gene responsible for resistance to ampicillin is primarily a gene from *Haemophilus influenzae* PBP3 conferring resistance to beta-lactam antibiotics.

al. 2023). Research on non-typhoidal *Salmonella* (NTS) isolates in Taiwan also revealed high resistance to azithromycin, which was associated with complex resistance mechanisms (Chiou et al. 2023). In Vietnam, *Salmonella* isolates from both vegetable and water samples exhibited high resistance to tetracycline (Nguyen et al. 2021a). The occurrence of AMR, which was also reported by the EU, demonstrated a notably high resistance to ampicillin and tetracycline in *Salmonella* isolates from humans and food-producing animals (Roasto et al. 2023, EFSA 2024). Furthermore, in addition to resistance, a high proportion of *Salmonella* isolates displayed intermediate resistance to ciprofloxacin and gentamicin antibiotics. These findings are in line with previous studies. For instance, studies on *S. Typhi* isolates from Cambodian children demonstrated high levels of intermediate resistance and re-

sistance to the antibiotic ciprofloxacin (Emary et al. 2012, Chheng et al. 2013). As Reed et al. (2019) reported in a review, *Salmonella* spp. isolates from humans exhibited a high resistance rate to ciprofloxacin. Nonetheless, the antibiotics included in this study remained effective in inhibiting the growth of the majority of *Salmonella* isolates, suggesting that they may still be viable options for treating *Salmonella* infections.

WGS data analysis using the SeqSero 2 tool has proved to be a highly effective approach, offering greater accuracy in serotype predictions than traditional serotyping methods (Cooper et al. 2020). Sequence analysis detected 32 serotypes among 81 *Salmonella* isolates, with the six most frequently identified serotypes being *S. Corvallis*, *S. Haifa*, *S. Weltevreden*, *S. Agona*, *S. Kentucky*, and *S. Livingstone*. Previous

research has identified *Salmonella* isolates as *S. Corvallis*, sourced from environmental samples among informal Cambodian markets (Schwan et al. 2022). *Salmonella* Haifa had been reported as one of the most commonly found serovars among poultry meat and farm samples in both Ethiopia and Nigeria (Dagnew et al. 2020, Raji et al. 2021, Abayneh et al. 2023). *Salmonella* Agona was identified as the most prevalent non-typhoidal serovar in chicken meat, while *S. Kentucky* was one of the serovars exhibiting high MDR (Tay et al. 2019). In recent years, *S. Kentucky* and *S. Livingstone* have become increasingly detected in poultry as well as poultry products (Guillén et al. 2020, Quinn et al. 2023). Interestingly, our study revealed the occurrence of *S. Weltevreden* in vegetables sampled from both farms and local markets, providing valuable insight into the potential connection between farm-level and market-level contamination. Moreover, *S. Weltevreden* was identified from sampled geckos due to the wild geckos being considered as the natural reservoir of serotype, indicating that the natural reservoir possibly influences the prevalence of *S. Weltevreden* among agricultural products (Nguyen et al. 2021b). *S. almonella* Weltevreden has also been identified as a serotype linked to human diarrhea and is commonly found in both food and environmental sources (Zhang et al. 2023). A study investigating pig and pork samples from the Cambodian border identified *S. Rissen* and *S. Anatum* as the most common *Salmonella* serotypes (Lay et al. 2021), both of which were also detected in the present study. Furthermore, the study identified two serotypes, *S. Hvitittingfoss* and *S. Thompson*, in the vegetable samples aligns with findings from another study on the distribution of *Salmonella* serotypes in Cambodian vegetable supply chains across the Siem Reap and Battambang provinces (Salazar et al. 2025). These findings indicated a high diversity of *Salmonella* serotypes in fresh food products in local markets, suggesting potential variations in transmission pathways across different Cambodian food supply chain stages.

Prediction of ARGs using the CARD database revealed that *Salmonella* isolates carried a diverse range of resistance genes, with MDR genes present in almost all analyzed isolates. Among the *Salmonella* sequences, 83% (67 out of 81) exhibited the *CPR* gene, which contributes to resistance against antibiotic classes such as macrolides, fluoroquinolones, and penams. *CPR* is a resistance-nodulation-cell division antibiotic efflux pump that plays a crucial role in MDR among Gram-negative bacteria (Fernando and Kumar 2013, Yamasaki et al. 2023). The ARGs associated with azithromycin resistance include *mphA*, *mef(B)*, *Mrx*, *E. coli emrE*, *efmA*, and *CPR*, with the latter being a key gene contributing to resistance to this antibiotic. Most of the genes detected in this study have also been described in other studies. The gene *mph(A)* is one of the main genes responsible for azithromycin resistance among sick children in China, and from food-producing animals and meat in Europe (Wang et al. 2023a, Ivanova et al. 2024). ARGs associated with resistance to tetracyclines include *tet(A)*, *tet(B)*, *tet(I)*, *tet(L)*, *tet(M)*, *tet(45)*, *tet(X4)*, *tetR*, and *emrK*. The *tet* gene family is the most prominent among *Salmonella* isolates from food samples and is associated with an efflux pump for tetracycline resistance (Maka and Popowska 2016, Boraei-Nexhad et al. 2023). In addition to this, the study also identified several genes responsible for beta-lactam resistance, including *ACC-1a*, *TEM-1*, *TEM-176*, *TEM-215*, *CMY-159*, *CMH-3*, *CTX-M-55*, *CTX-M-65*, *LAP-2*, *OXA-1*, *OXA-10*, *Sed-1*, *SHV-11*, *SHV-26*, and *LptD*. The *ACC*, *TEM*, *CMY*,

CMH, *LAP*, *OXA*, *Sed*, and *SHV* genes are associated with antibiotic inactivation mechanisms, whereas *LptD* is involved with the ATP-binding cassette antibiotic efflux pump. Several studies reported that the beta-lactamase genes (*bla*) influence resistance to the beta-lactam class of antibiotics. For instance, ~77% (33 out of 43) of NTS isolates from humans and animals in central Ethiopia carried the *blaTEM* genes (Egualé et al. 2017). Another study on *Salmonella* isolates from poultry, poultry products, and humans also identified the presence of *bla* genes, such as *blaTEM*, *blaCTX*, *blaSHV*, and *blaACC* genes (Hasman et al. 2005). In addition to the ARGs mentioned above, the same study also identified numerous resistance genes responsible for resistance mechanisms to other tested antibiotics. These findings highlight the extensive diversity of ARGs among *Salmonella* isolates from fresh food products in Cambodia.

Moreover, MDR phenotypes were predominantly detected in isolates from meat and vegetables collected at local markets, whereas only two isolates originated from farm samples. However, fewer samples were collected from farms compared to markets. Regarding genotype data, most isolates carried MDR genes. The most common MDR genes were *sdhA*, *marA*, *acrB*, *rsmA*, *mdtM*, *golS*, *mdsA*, *mdsB*, and *mdsC*. These MDR genes are associated with antibiotic efflux pumps and reduced the permeability of the bacterial cell wall to antibiotics. Several studies have shown an increase in MDR among *Salmonella* isolates. Approximately 38% of *Salmonella* serovars isolated from humans and animals in a study from India exhibited MDR (Borah et al. 2022). Research on zoonotic *Salmonella* isolates in Bangladesh revealed that up to 94% of those from broiler chickens were MDR (Das et al. 2022). Furthermore, all *Salmonella* isolates from the raw milk of healthy dairy cows in China exhibited MDR, with over 60% carrying the efflux pump genes *oqxA* and *oqxB*, which were also identified in a previous study (Liu et al. 2022). There are clear linkages between farms and markets, which may explain transmission of resistant bacteria in the food production chain. For example, lack of awareness and implementation of appropriate hygiene and sanitation practices, poor food storage and handling conditions, and high and unstable temperatures in Cambodian local markets all contribute to bacterial growth and cross-contamination (Huoy et al. 2024). The wide variety of MDR genes identified in this study necessitates a deeper understanding of their resistance mechanisms to enhance monitoring and control efforts against the spread of MDR *Salmonella* in Cambodia's food value chain.

The observed phenotypic and genotypic patterns of *Salmonella* AMR included resistance to most of the antibiotic classes, except for amoxicillin resistance. Our study showed that there was a high matching percentage between phenotypic and genotypic resistance, indicating that phenotypic resistance profiling is a useful tool when no detailed characterization is needed. A strong association was observed between AMR phenotypes and specific ARGs across antimicrobial classes such as phenicols, tetracycline, quinolones, aminoglycosides, and folate pathway antagonists class. For example, all *Salmonella* isolates with resistance to phenicols were aligned with the ARG-identifying genes such as *cmlA1*, *cmlA5*, *flor*, *rsmA*, *catB3*, *mdsA*, *mdsB*, *mdsC*, *golS*, and *mdtM*. However, a complete match between phenotypic and genotypic resistance was not always observed, as was the case with amoxicillin, which had only a 24% matching percentage. Similarly, a study on *Salmonella* serovars Derby and Rissen from the pig

value chain in Vietnam found a lack of concordance between AMR phenotypes and genotypes (González-Santamarina et al. 2021). The observed mismatches in our study may be attributed to limitations of the CARD database, as well as incomplete gene annotations, absent regulatory elements, or strain-specific mutations that affect gene expression rather than gene presence. To improve detection and validation, future studies could incorporate complementary tools such as Abricate and AMRFinderPlus.

The present study uncovered a high diversity of serotypes among *Salmonella* isolates as well as a high prevalence of AMR. The results emphasize the potential role of fresh food products in the widespread dissemination of *Salmonella* strains resistant to multiple antibiotics. This is likely associated with the unrestricted use of antibiotics in the livestock sector and poor hygiene and sanitation practices along the entire chain from production to consumption. The WGS data provided a deeper insight into the *Salmonella* resistance genes responsible for the MDR mechanisms. This study underscores the need for a control strategy to reduce levels of antibiotic resistance in *Salmonella* in the food value chain.

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Author contributions

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

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Data availability

Data will be made available on request.

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This thesis investigated the prevalence of *Salmonella* and its antimicrobial resistance (AMR) in Cambodian fresh food products collected from local markets and farms in Cambodia. The results showed that 48% of the samples were contaminated with *Salmonella*, exhibiting both a high rate of AMR and considerable genetic diversity among the isolates. These findings highlight a significant public health risk and emphasize the need for effective control strategies to eliminate these highly virulent and resistant strains from the food chain.

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