Journal of Applied Microbiology, 2025, 136(6), lxaf137
https://doi.org/10.1093/jambio/lxaf137
Advance access publication date: 3 June 2025
Research Article

JOURNAL OF APPLIED MICROBIOLOGY
OXFORD UNIVERSITY PRESS

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, Tibebu 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-

Received 28 March 2025; **revised** 26 May 2025; **accepted** 2 June 2025

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										
	Pork	Beef	Chicken	Fish	Seafood	Bok choy	White cabbage	Salad	Morning glory	Curly cabbage	Total[a]
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

[a]These 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, fluoroquinolones, 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

[a]Monobactam; carbapenems; cephalosporin; and penicillin are subclasses of beta-lactam antibiotics.
[b]Intermediate 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 = amoxycillin.

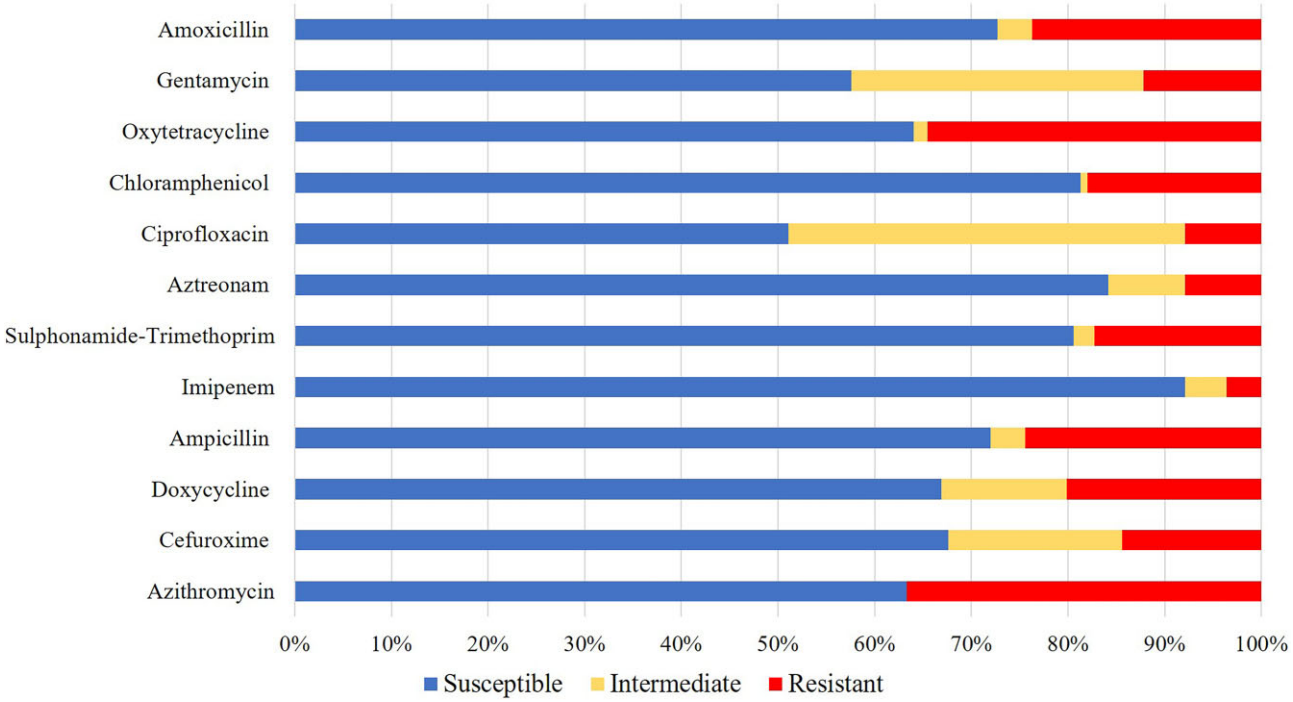

Figure 1. Percentage (%) of antimicrobial susceptibility exhibited among *Salmonella* spp. ($n = 139$), isolated from different food commodities collected in Cambodia, for each of the 12 included antibiotics.

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 detecteamong 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 (%)				
			Pork/beef	Poultry meat	Seafood/fish	Vegetable	Total
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)
D	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*. Hvittingfoss	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)

[a]The 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).
[b]The antigenic formula is possibly closely related to the strain *S*. Annedal (16: r, i: e, n, x).

copeptides, lincosamides, nucleosides, peptides, phosphonic acid, pleuromutilins, 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. Hvittingfoss (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. Hvittingfoss (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		

[a]AMR 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 = amoxycillin.
[b]Antimicrobial classes: macrolide (Azm), cephalosporing (Cxm), tetracycline (Do, Ot), penicillin (Amp, Aml), carbapenems (Ipm), sulfonamide/trimethoprim (Sxt), monobatams (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	*tet(A)*, *tet(B)*, *tet(J)*, *tet(L)*, *tet(M)*, *tet(45)*, *tet(X4)*, *tetR*, *emrK*
Aminoglycoside	*AAC(3)-IId*, *AAC(3)-IIe*, *AAC(3)-IVa*, *AAC(6′)-Iaa*, *AAC(6′)-Ib10*, *AAC(6′)-If*, *AAC(6′)-Ii*, *AAC(6′)-Iid*, *AAC(6′)-Iy*, *aadA*, *aadA2*, *aadA3*, *aadA7*, *aadA16*, *aadA23*, *acrD*, *APH(3′)-Ia*, *APH(3′′)-Ib*, *APH(4)-Ia*, *APH(6)-Id*, *baeR*, *baeS*, *cpxA*, *kdpE*, *mdtA*, *mdtB*, *mdtC*
Quinolone/fluoroquinolone	*emrA*, *emrB*, *emrR*, *MdtK*, *QepA2*, *QnrB12*, *QnrB19*, *QnrS1*, *QnrS2*, *QnrD1*, *gyrA*, *gyrB*, *parC*, *adeF*
Phenicol	*floR*, *catA4*, *catB3*, *cmlA1*, *cmlA5*, *catII from E. coli K-12*
Sulfonamide–trimethoprim	*sul1*, *sul2*, *sul3*, *dfrE*, *dfrA1*, *dfrA12*, *dfrA14*
Macrolide	*mphA*, *mef (B)*, *Mrx*, *E. coli emrE*, *efmA*, *CR P*
MDR genes	*sdiA*, *marA*, *rsmA*, *ramA*, *mdtM*, *oqxA*, *oqxB*, *acr B*, *Ac r E*, *Acr F*, *AcrS*, *fosA5*, *acrA*, *AcrAB-TolC with A c rR mu t ation*, *AcrAB- T olC with MarR mutation s*, *E. coli soxS mutation*, *E. coli sox R muta tion*, *K. pneumoniae acrR mutati on*, *CRP*, *efrA*, *ErmB*, *evgA*, *gadW*, *H-NS*, *mdtE*, *msrC*, *KpnE*, *KpnF*, *KpnG*, *Kp nH*, *Md tQ*, *golS*, *mdsA*, *mdsB*, *mdsC*, *K. pneumoniae OmpK37*, *E. coli mdfA*
Disinfecting agents and antiseptics	*qacG*, *qacL*, *qacEdelta1*
Other ARGs	*ArnT*, *bacA*, *eptB*, *FosA2*, *FosA6*, *FosA7*, *FosA8*, *mdtG*, *OmpA*, *PmrF*, *ugd*, *MCR-1.1*, *E. coli GlpT mutation*, *E. coli UhpT mutation*, *msbA*, *eatAv*, *vanG*, *vanY gene in (vanA*, *vanB*, *vanF*, *vanM) cluster*, *vanT gene in vanG cluster*, *vanXY gene in vanC cluster*, *ln uA*, *lsaA*

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
	Cephem (cephalosporin)	Cefuroxime (Cxm)	18	18[a]	100 00
	Penicillin	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
Phenicols	-	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.
[a]MDR 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).
[b]Resistance 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 resistance 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.* Hvittingfoss 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(J)*, *tet(L)*, *tet(M)*, *tet(45)*, *tet(X4)*, *tet*R, 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 (Mąka 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 *Lpt*D. 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 *bla*TEM genes (Eguale et al. 2017). Another study on *Salmonella* isolates from poultry, poultry products, and humans also identified the presence of *bla* genes, such as *bla*TEM, *bla*CTX, *bla*SHV, and *bla*ACC 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 *sdiA*, *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.

Acknowledgments

The authors express their gratitude to SciLife Lab Uppsala, Sweden, for offering sequencing services essential to this project. We would like to acknowledge the Bioinformatics Core Facility, Faculty of Medicine and Health Sciences and Clinical Genomics Linköping, SciLife Laboratory, Department of Biomedical and Clinical Sciences, Linköping University, for assistance with bioinformatic analyses. They also sincerely thank the Higher Education Improvement Project (HEIP), funded by the Royal Government of Cambodia through the Ministry of Education, Youth, and Sport, for supporting the microbiology laboratory facilities at RUPP, enabling sample processing.

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)

Conflict of interest: There is no conflict of interest declared.

Funding

This work was supported by the Swedish International Development Cooperation Agency (SIDA) through the Sweden-Royal University of Phnom Penh Bilateral Program (contribution number 11599).

Data availability

Data will be made available on request.

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Received 28 March 2025; **revised** 26 May 2025; **accepted** 2 June 2025