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Characterization of Antimicrobial Resistance Profiles and Extended-Spectrum Beta-Lactamase Resistance Genes in *Salmonella* Isolates from Different Food Matrices in Cambodia

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Abstract: *Salmonella is the most common cause of salmonellosis, a foodborne infection that is transmitted by consuming contaminated food. It is becoming more prevalent worldwide, particularly in Cambodia. Additionally, Salmonella is recognized as one of the drug-resistant bacteria, posing serious public health problems that spread quickly due to the misuse and overuse of antibiotics in healthcare, livestock husbandry, and agriculture. Therefore, the purpose of this study was to determine the occurrence of Salmonella from different food matrices in Cambodia and to assess its profile of antibiotic resistance, including the detection of extended-spectrum beta-lactamase (ESBL) resistant genes. From January to December 2023, a total of food samples (n=1,506), categorized into twenty-two sample types, were tested for Salmonella using the standard method ISO 6579-1:2017. The positive Salmonella were subjected to antimicrobial susceptibility testing by using disc diffusion method against 22 antibiotics following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. The ESBL-producing Salmonella were screened for the resistance genes via conventional PCR. The results showed that Salmonella was present in 2.7% (40/1506) of the analyzed food samples originated from seafood (10.3%), fish and fish products (13.8%), and meat and meat products (28.7%). Antimicrobial susceptibility assay revealed that 57.5% (23/40) were resistant to amoxicillin, piperacillin, and ticarcillin, and 52.5% (21/40) were resistant to nalidixic acid, while 35% (14/40) of the Salmonella isolates were susceptible to all of the antibiotics tested. It was also found that 45% (18/40) of Salmonella isolates were multidrug-resistant (MDR) and 27.5% (11/40) were ESBL-producing strains originated from all samples of meat and meat products. Molecular detections revealed that 45.5% of the ESBL-producing Salmonella isolates carried bla $_{CTX-M}$ group 9 genes. Our findings demonstrated the primary sources of Salmonella contamination, including seafood, fish, and meat, and meat products serving as a significant reservoir for multidrug-resistant (MDR) and ESBL-producing strains. This pathogen's emergence highlights the need for regulatory measures intended to restrict the use of antibiotics in food agriculture. As a recommendation, further study should employ other PCR primers to determine the remaining ESBL-producing strains in combination with sequencing to define the specific resistance genes, or whole genome sequencing, to comprehensively identify resistance genes and trace the spread of resistant Salmonella strains in the food production environment.*

Keywords: *Salmonella*; food; antibiotic resistance; Extended-spectrum beta-lactamase; Cambodia

1. INTRODUCTION

Salmonella is a rod-shaped, Gram-negative bacterium that is a member of the *Enterobacteriaceae* family. Its two primary species are *Salmonella enterica* and *Salmonella bongori*. To date, *Salmonella* has been found to contain over 2,500 different serotypes [1]. *Salmonella* is the most common cause of salmonellosis, a foodborne infection that is transmitted by consuming contaminated food. In most cases, salmonellosis is caused by contaminated food products, particularly those of animal origin such as poultry, eggs, beef, and pork. Fruits and vegetables also have been reported as vehicles in *Salmonella* transmission, and contamination can

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occur at multiple steps along the food chain [2]. Salmonellosis remains one of the most frequent food-borne diseases worldwide, especially in developing countries [3]. It is frequently characterized by symptoms like diarrhea, vomiting, nausea, fever, and stomach pain. According to estimates from the Centers for Disease Control and Prevention (CDC), foodborne *Salmonella* infections result in around 1.35 million cases annually in the US, 26,500 hospitalizations, and 420 fatalities [1].

Salmonella is considered one of the drug-resistant bacteria that rapidly spread as a result of the misuse and overuse of antibiotics in agriculture, livestock husbandry, and human medicine. The usage of antibiotic growth promotions (AGPs) in the production of food animals has specifically been linked to the emergence of multidrug-resistant (MDR) *Salmonella* strains. AGPs were first raised up in the mid-1950s, and for many years thereafter, and the experts suspected that AGP usage could contribute to the development of antibiotic resistance. Notably, farms using AGPs were found to harbor more resistant bacteria than those without AGPs. For instance, a study in 1975 depicted that administering low-dose oxytetracycline in chicken feed not only colonized poultry with tetracycline-resistant and other drug-resistant *E. coli*, but also caused acquisition of resistance of *E.* coli among the farm workers. The distribution of resistance might be due to direct or indirect contact through food, water, and animal waste applied to farm fields, where horizontal gene transfer of plasmids, conjugation mechanism, could be possible [4]. Interestingly, studies on *Aeromonas salmonicida* from both fish and humans showed high-frequency transfer of their resistance plasmids containing class I integrons to *E. coli* and *Salmonella* [5]. Antimicrobial resistance is a major threat to global health concern with causing an estimated 1.27 million deaths and contributing to 4.95 million deaths worldwide [6]. The subtherapeutic or preventative use of antibiotics in food animals is a significant leading cause of the rise of antibiotic-resistant *Salmonella*. This practice encourages the development of antimicrobial-resistant strains on farms, significantly increasing the risk to human health from consuming contaminated animal products [7]. Additionally, MDR *Salmonella* has been found in vegetables, likely due to contamination from irrigation water [8].

A particularly concerning development is the emergence of Extended-Spectrum Beta-Lactamase (ESBL)-producing *Salmonella enterica*, which exhibits resistance to beta-lactam antibiotics, including third-generation cephalosporins. Cephalosporins are commonly used to treat invasive *Salmonella* infections, and resistance to these drugs poses a serious threat to public health. Over the past two decades, ESBL-producing *Salmonella* strains have emerged among animals and animal food products, raising concerns about their potential transmission to humans through contaminated food [9]. Previous work in Cambodia has documented a high prevalence of *S. enterica* contamination (80%) among retail meats sold in Phnom Penh markets [10]. Another study in Cambodia found ESBL-producing *Salmonella* in pork (15/60; 25%), fish (10/60; 17%), and chicken (1/30; 3%). The *bla* $_{\text{CTX-M}}$ group 1 genes, specifically *bla* $_{\text{CTX-M-55}}$, were the most common ESBL type in all sample types [11]. While previous studies have focused on specific food sources, there is limited research on the prevalence of *Salmonella* and its antimicrobial-resistant profile in different food matrices in Cambodia. Therefore, this study aims to determine the prevalence of *Salmonella* and its antibiotic resistance profile, including identifying resistance genes of ESBL-producing *Salmonella* isolated from various food matrices in Cambodia.

2. METHODOLOGY

2.1 Food sample collection

Between January and December 2023, 75 companies submitted a total of 1,506 samples from diverse sectors, including hotels, food catering services, restaurants, food industry, import and export companies, and individuals. Some clients, including restaurants, hotels, and catering services, submitted samples on a regular schedule followed their weekly, monthly, quarterly, or semi-annual food testing schedules. While import and export businesses submitted samples for yearly testing in accordance with certification or export regulations. Food industrial companies regularly tested both raw materials and final packaged products as part of internal quality assurance. Ad hoc sample submissions were made by individuals, typically as a result of personal food safety concerns. The dataset represents actual monitoring practices rather than a representative sampling frame because these were client-submitted samples. Approximately 50 g of each sample was collected in a sterile plastic pouch and transported to the Laboratory of Environment and Food Safety (LEFS) of Institut Pasteur du Cambodge (IPC), located in Phnom Penh, Cambodia. Perishable products need to be refrigerated at 2-8 °C during transport and the analysis was performed within 24 hours after collection. Food sample types are detailed in Table 1.

Table 1. Collected food samples during the period from January to December 2023

No.	Sample Types	Number of Sample
1	Fresh pasta	2
2	Raw egg	2
3	Honey	3
4	Fresh fruit/vegetable	8
5	Spice	20

6	Dried fruit/herb	20
7	Sauce	22
8	Milk and milk products	23
9	Alcoholic drink	24
10	Fruit juice	26
11	Additive/Ingredient	27
12	Ice cream	28
13	Baby foods	31
14	Pastries/dessert	35
15	Soft drink	36
16	Seafood	39
17	Industrial pastries/biscuits	74
18	Fish and fish products	80
19	Meat and meat products	87
20	Ready-to-eat foods	125
21	Cooked foods	372
22	Instant products	422
	Total	1506

2.2 Salmonella detection

The samples were analysed using the standard method, ISO 6579-1:2017. The amount of 25g of sample was first pre-enriched in 225 mL of buffered peptone water (BPW), then incubated at 37 °C for 18 hours. After the incubation, 0.1ml and 1ml were inoculated into 10ml of Rappaport-Vassiliadis medium (RVs) and 10 ml of Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn), respectively. Then RVs broth was incubated at 41.5 °C for 24 h and the MKTTn broth at 37 °C for 24 h. Each culture from selective enrichment broth was plated out on two selective solid media of Xylose Lysine Deoxycholate agar (XLD) and RAPID' *Salmonella* chromogenic agar (R'SAL), then incubated at 37 °C for 24 h. Lactose-negative colonies on XLD and magenta colonies on R'SAL were selected for confirmation by using MALDI Biotyper®. All positive *Salmonella* strains were stored in skim milk medium with glycerol at -20 °C for further studies.

2.3 Antimicrobial susceptibility testing

All *Salmonella* isolates were tested for antimicrobial susceptibility using the disc diffusion method according to the recommendation of the 2024 European Committee on Antimicrobial Susceptibility Testing (EUCAST) [12]. Twenty-two commercial antibiotic discs from eight different classes were tested such as (1) Penicillins: amoxicillin (AMO; 20µg), amoxicillin-clavulanic acid (AMC; 20/10µg), piperacillin (PIL; 30µg), piperacillin-tazobactam (PTZ; 30/6µg), ticarcillin (TIC; 75µg), ticarcillin-clavulanic acid (TCC; 75/10µg), (2) Cephalosporins: cefepime (FEP; 30µg), cefotaxime (COX; 5µg), cefoxitin (FOX; 30µg), ceftazidime (CZD; 10µg), (3) Carbapenems: ertapenem (ETP; 10µg), imipenem (IPM; 10µg), (4) Monobactams: aztreonam (ATM; 30µg), (5) Fluoroquinolones: ciprofloxacin (CIP; 5µg), levofloxacin (LVX; 5µg), nalidixic acid (NAL; 30µg), (6) aminoglycosides: amikacin (AKN; 30µg), gentamicin (GMN; 10µg), tobramycin (TMN; 10µg), (7) Miscellaneous: fosfomycin (FOS; 200µg), cotrimoxazol (SXT; 1.25/23.75µg), (8) Tetracyclines: tigecycline (TGC; 15µg). *Escherichia coli* ATCC 25922 was used as the quality control. The results were interpreted according to the recommendation of the EUCAST breaking point table [13]. The strains were considered multi-drug resistant (MDR) if they demonstrated resistance to three or more classes of antibiotics (at least one antibiotic of each class) [14]. ESBL-producing strains were suspected when the inhibition zone around any cephalosporin increased toward the central disk with amoxicillin-clavulanic acid [15].

2.4 ESBL genes detection

Salmonella DNA was extracted using the GENCLEAN Turbo® 96 Kit (vacuum protocol). Isolated colonies were suspended in 1500 µL of nuclease-free water and subjected to boiling in the water bath at 95 °C for 10 minutes. DNA extraction was then carried out following the manufacturer's vacuum protocol instructions. Finally, the extracted DNA was transferred into a new microtube and stored at -20 °C until use. Extracted DNA was amplified for *bla* genes, starting with *bla* $_{CTX-M}$ group 1. If negative, we continued PCR with *bla* $_{CTX-M}$ group 9, *bla* $_{TEM}$, and *bla* $_{SHV}$, which are listed in Table 2, with 30 µl total of mixing reagents, followed by 1 X of Taq Buffer, 2.5 mM of MgCl2, 2 mM of dNTP, 0.66 mM of both primers, 0.08 U DNA Polymerase, and 5 µl of DNA template. The PCR mixture was amplified using Bio-Rad T100, an initial step at 94 °C for 5 min, followed by 32 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 1 min, with a final step at 72 °C for 10 min. All PCR amplicons were separated by gel electrophoresis on a 1% (wt/vol) Agarose gel. Staining of the gel was conducted with 10,000X GelRed.

Table 2. Sequences of primers used for the detection of *bla* genes

Gene	Primer	Sequence 5' to 3'	Size (bp)
bla $_{CTX-M}$ Group 1	ISEcpPROM+	5'-tgc-tct-gtg-gat-aac-ttg-c-3'	1155
	CTXMpréB	5'-cac-ttt-gcc-gtc-gtc-taa-ggc-g-3'	
	ISEcpPROM-	5'-gca-gtc-taa-att-ctt-cgt-g-3'	1041
	CTXMpréB	5'-cac-ttt-gcc-gtc-gtc-taa-ggc-g-3'	

bla CTX-M Group 9	ISEcpPROM+ IS903Bint	5'-tgc-tct-gtg-gat-aac-ttg-c-3' 5'-gct-ttt-tga-ctt-tcc-act-cgc-3'	1587
	ISEcpPROM- IS903Bint	5'-gca-gtc-taa-att-ctt-cgt-g-3' 5'-gct-ttt-tga-ctt-tcc-act-cgc-3'	1473
	ISEcpPROM+ IS903B2	5'-tgc-tct-gtg-gat-aac-ttg-c-3' 5'-ggc-gta-agc-tgc-atc-tgg-3'	1629
	ISEcpPROM- IS903B2	5'-gca-gtc-taa-att-ctt-cgt-g-3' 5'-ggc-gta-agc-tgc-atc-tgg-3'	1515
bla CTX-M	CTX-MA1 CTX-MA2	5'-scs-atg-tcg-agy-acc-agt-aa-3' 5'-ccg-cra-tat-grt-tgg-ttg-tg3'	450
bla TEM	PRETEM-1 PRETEM-2	5'-gta-tcc-gct-cat-gag-aca-ata-3' 5'-tct-aaa-gta-tat-atg-agt-aaa-ctt-ggt-ctg-3'	955
bla SHV	SHV-A SHV-B	5'-atg-cgt-tat-wtt-cgc-ctg-tgt-3' 5'-tta-gcg-ttg-cca-gtg-ctc-g-3'	860

3. RESULTS AND DISCUSSION

3.1 Prevalence of Salmonella in food matrices

Microbiological examination revealed that *Salmonella* was present in 2.7% (40/1506) of the analyzed food samples. Three out of 22 food sample types examined were positive with *Salmonella*, including seafood (10.3%), fish and fish products (13.8%), and meat and meat products (28.7%). *Salmonella* was not found among the remaining 19 food sample types. This study examined 1,506 food samples in total, covering a wide variety of product categories frequently present in the regional food supply chain. Instant products represented the largest proportion of these samples (n=422), followed by cooked foods (n=372) and raw and ready-to-eat foods (n=125). These high sampling numbers may be a result of widespread consumption and frequent client submissions. Meat and meat products (n=87), fish and fish products (n=80), and industrial pastries/biscuits (n=74) are other significant categories that have notable representation. The wide range of food samples examined highlights how thorough this monitoring effort is, providing a comprehensive evaluation of microbiological safety for both processed and unprocessed food products. Table 3 reports the prevalence of *Salmonella* isolates from different categories of food.

Table 3. *Salmonella* prevalence in different food samples during Jan – Dec 2023

Sample Types	Number of Samples	
	Analyzed	Positive
Fresh pasta	2	0 (0.0%)
Egg	2	0 (0.0%)
Honey	3	0 (0.0%)
Fresh fruit/vegetable	8	0 (0.0%)
Spice	20	0 (0.0%)
Dried fruit/herb	20	0 (0.0%)
Sauce	22	0 (0.0%)
Milk and milk products	23	0 (0.0%)
Alcoholic drink	24	0 (0.0%)
Fruit juice	26	0 (0.0%)
Additive/Ingredient	27	0 (0.0%)
Ice cream	28	0 (0.0%)
Baby foods	31	0 (0.0%)
Pastries/desserts	35	0 (0.0%)
Soft drink	36	0 (0.0%)
Seafood	39	4 (10.3%)
Industrial pastries/biscuits	74	0 (0.0%)
Fish and fish products	80	11 (13.8%)
Meat and meat products	87	25 (28.7%)
Raw and ready-to-eat foods	125	0 (0.0%)
Cooked foods	372	0 (0.0%)
Instant products	422	0 (0.0%)
Total	1506	40 (2.7%)

The findings of this study indicated that *Salmonella* contamination in food matrices is primarily associated with meat, fish, and seafood. Meat and meat products had the greatest percentage of *Salmonella* (25/87; 28.7%), which is similar to other studies that show a high prevalence of this pathogen in raw meats because of its ability to survive in handling conditions [9], [10]. Moreover, the contamination rates of *Salmonella* in fish and fish products (11/80; 13.8%) and seafood (4/39; 10.3%) were notably lower compared to meat and meat products. Previous findings in Cambodia have been reported *Salmonella* contamination rates of 71% (53/75) in meat, 64% (32/50) in seafood, and 33% (53/160) in leafy green vegetables [16]. Other studies in Cambodia have reported high prevalence of *Salmonella* contamination. Lay *et al.* [10] found *S. enterica* in 88.2% of retail meats, while

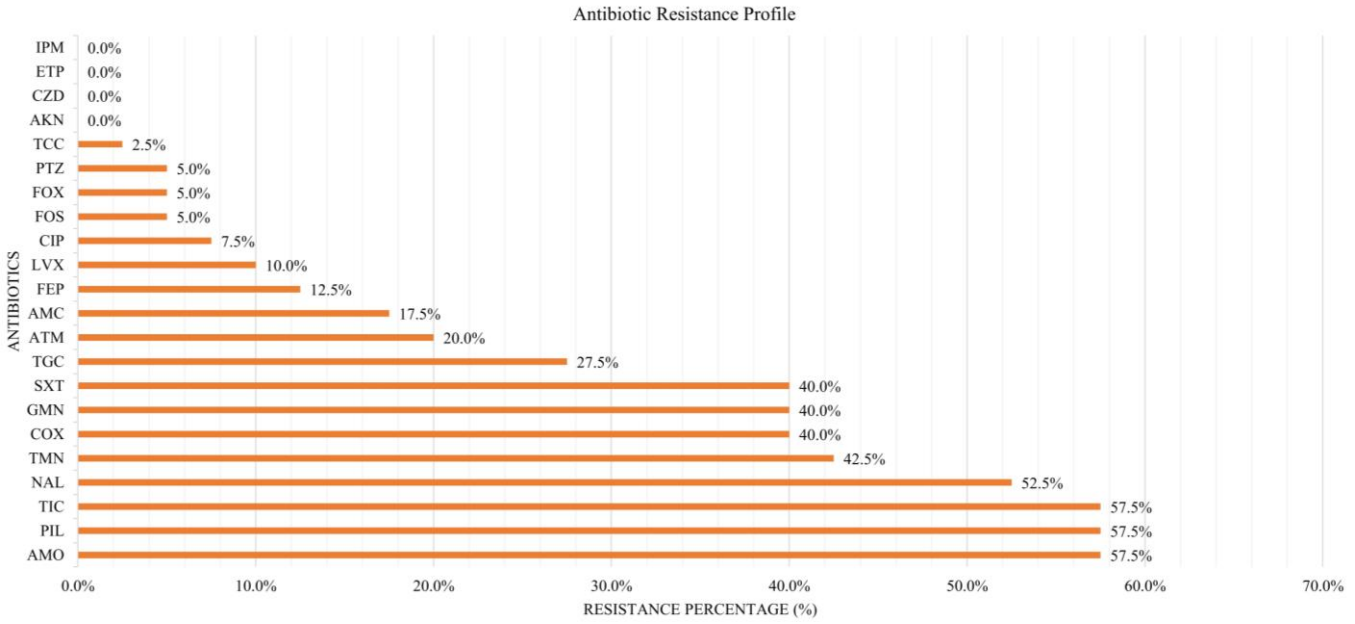

Fig. 1. Acquired percentage of antimicrobial susceptibility testing in *Salmonella* isolates from meat and meat products, fish and fish products, and seafood. IPM, imipenem; ETP, ertapenem; CZD, ceftazidime; AKN, amikacin; TCC, ticarcillin-clavulanic acid; PTZ, piperacillin-tazobactam; FOX, cefoxitin; FOS, fosfomycin; CIP, ciprofloxacin; LVX, levofloxacin; FEP, cefepime; AMC, amoxicillin-clavulanic acid; ATM, aztreonam; TGC, tigecycline; SXT, cotrimoxazol; GMN, gentamicin; COX, cefotaxime; TMN, tobramycin; NAL, nalidixic acid; TIC, ticarcillin; PIL, piperacillin; AMO, amoxicillin.

Nadimpalli *et al.* [11] reported ESBL-*Salmonella* prevalence of 25% in pork (15/60) and 17% in fish (10/60) from Phnom Penh markets. The detected contamination of meat, fish, and seafood might be linked to the presence of *Salmonella* in slaughterhouses, cross-contamination during processing due to repeated use of materials in each process, use of contaminated water, and improper storage conditions. To prevent such contamination, producers and processors should comply GMP/HACCP standards. Hotels, restaurants, or catering services should considerably choose verified supplier, ensure proper sanitation in food processing, especially by dividing the raw and ready-to-eat foods, and enhance knowledge in food hygiene and temperature controls for their staffs. In addition to preventive measures, the detection of *Salmonella* from environmental, materials, consumables, and water used during food preparation from supplier and hotels or catering should also be considered to identify the cause of contamination, since the presence of *Salmonella* in the environment and carcasses in slaughterhouses has a high genetically associated [17]. Moreover, equipment used in slaughterhouses, such as cutting boards, has also been found to have contaminated with *Salmonella* [18].

Salmonella was not found in the other 19 food categories indicates that these three sample types (meat, fish, and seafood) are more susceptible to bacterial contamination, possibly as a result of cross-contamination, incorrect handling, or insufficient cooking temperatures. Since meat, fish, and seafood samples were the products most often identified in the spreading of *Salmonella,* monitoring of these products is the key point in preventing and controlling the spread of this pathogen, as well as in providing healthier food products.

3.2 Antimicrobial resistance patterns of Salmonella isolates

Out of 40 *Salmonella* isolates for antimicrobial susceptibility testing were demonstrated variable resistance levels. Figure 1 shows the antibiotic resistance profile corresponding percentage in each drug. The most common resistance observed was to amoxicillin, piperacillin, and ticarcillin (57.5%, n=23), followed by nalidixic acid (52.5%, n=21). Additionally, 40% (n=16) of isolates exhibited resistance to cefotaxime (a third-generation cephalosporin), cotrimoxazole (a folate synthesis inhibitor), and gentamicin (an aminoglycoside), which are antibiotics commonly used in human medicine. The moderate resistance levels were recorded for tigecycline (27.5%, n=11), aztreonam (20%, n=8), and amoxicillin-clavulanic acid (17.5%, n=7). Resistance rates were lower for fluoroquinolones, with levofloxacin at 10% (n=4), ciprofloxacin at 7.5% (n=3), 5% (n=1) to three antibiotics included cefoxitin, fosfomycin, and piperacillin-tazobactam, and 2.5% (n=1) to ticarcillin-clavulanic acid. Interestingly, none of the *Salmonella* isolates showed resistance to imipenem, ertapenem, ceftazidime, and

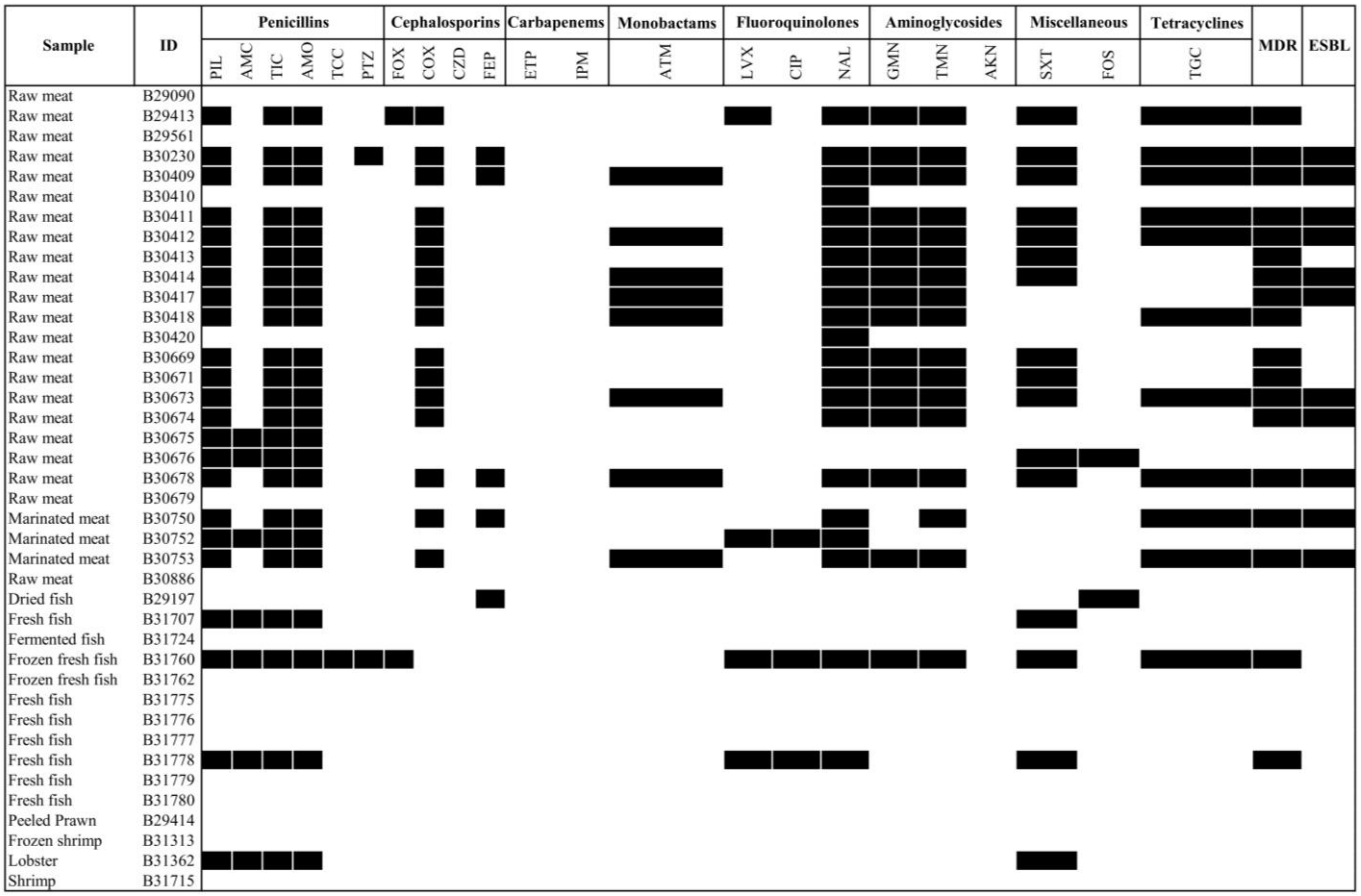

Fig. 2. Prevalence of antimicrobials resistance profiles and ESBL-producing *Salmonella*. Black squares denote the presence of antibiotic resistance, multi-drug resistance (MDR), and ESBL production.

amikacin, suggesting that these antibiotics are still effective treatment options. The antimicrobial resistance patterns observed in this study underscore the growing concern of antibiotic-resistant *Salmonella* strains. The high rates of resistance to β-lactam antibiotics, specifically to amoxicillin, piperacillin, and ticarcillin (57.5%), as well as to nalidixic acid (52.5%) and gentamicin (40%), are a result of the selection pressure carried on by the widespread application of antibiotics in both clinical and veterinary settings. The sub-therapeutic or preventative use of antibiotics in animals raised for food is probably the main cause of this resistance, as it encourages the evolution of resistant *Salmonella* strains and raises the possibility of human transmission through contaminated animal products [7].

The results of *Salmonella* antibiotic resistance by food source are presented in Figure 2. The drug susceptibility assay revealed that *Salmonella* isolates were susceptible to all antimicrobials in 35% (14/40), represented 16% (4/25) from meat and meat products, 63.6% (7/11) from fish and fish products, and 75% (3/4) from seafood. Twenty-six isolates (26/40; 65%) were resistant to at least one antibiotic, while eighteen isolates (18/40; 45%) exhibited multidrug resistance (MDR), defined as resistance to at least three antibiotic classes. MDR strains were predominantly found in meat and meat products (64%, 16/25), followed by fish and fish products (18%, 2/11), and seafood samples exhibited lower resistance to the antimicrobial agents tested in this study. It was also found that 27.5% (11/40) of *Salmonella* strains were extended-spectrum β-lactamase (ESBL)-producing. All *Salmonella*-producing ESBLs originated from meat and meat products at 100% (11/11). These results align with previous findings in Cambodia, where MDR *Salmonella* was frequently identified in retail meats, posing significant risks to food safety and public health [19]. Indeed, the prevalence of *Salmonella* MDR strains in Asian region are known as a major concern due to inadequate food handling, unsafe production practices, and lifestyle factors [20]. In China, MDR *Salmonella* strains have highly found in retail meats were 81% prevalence rate [21]. In Thailand, more than 50% of ESBL-producing *Salmonella* isolates were MDR strains [18]. Therefore, the presence of MDR *Salmonella* (45%) in the present study is particularly alarming, as it limits treatment options and poses significant public health risks. Our findings show that antibiotic-resistant *Salmonella* is highly prevalent in meat and fish products, leading to the need

for improved surveillance and stricter antimicrobial stewardship in Cambodia. The presence of MDR and ESBL-producing *Salmonella* poses a direct threat to human health, potentially leading to treatment failures and prolonged infections. To mitigate these risks, it is crucial to implement proper hygiene practices in food handling, enhance monitoring systems for antimicrobial resistance, and regulate the use of antibiotics in livestock and aquaculture [5].

Table 4. *Beta-lactamase resistance gene detection results*

ID Strains	Food	ESBL Producing	*bla* Gene Detected
B30230	Raw meat	+	-
B30409	Raw meat	+	*bla* $_{\text{CTX-M}}$ group 9
B30411	Raw meat	+	-
B30412	Raw meat	+	-
B30414	Raw meat	+	-
B30417	Raw meat	+	-
B30673	Raw meat	+	-
B30674	Raw meat	+	*bla* $_{\text{CTX-M}}$ group 9
B30678	Raw meat	+	*bla* $_{\text{CTX-M}}$ group 9
B30750	Marinated meat	+	*bla* $_{\text{CTX-M}}$ group 9
B30753	Marinated meat	+	*bla* $_{\text{CTX-M}}$ group 9

3.3 ESBL-producing Salmonella genes

Out of 11 phenotypically confirmed ESBL-producing *Salmonella* isolates recovered from raw and marinated meat (Table 4), PCR analysis detected *bla* $_{\text{CTX-M}}$ group 9 in five isolates (45.5%). No *bla* $_{\text{CTX-M}}$ group 1, *bla* $_{\text{TEM}}$, or *bla* $_{\text{SHV}}$ genes were detected in any isolates. Notably, all *bla*-positive isolates were derived from both raw and marinated meat, indicating gene distribution across meat and meat products. Although all 11 *Salmonella* isolates demonstrated phenotypic ESBL activity, resistance genes were only detected in five isolates using conventional PCR, suggesting the presence of other ESBL genes that were not targeted in this study. CTX-M-producing *Enterobacteriaceae* have been known to spread extensively in Southeast Asia and the Eastern Mediterranean region [22]. These CTX-M enzymes are primarily carried on plasmids, which are mobile genetic elements associated with horizontal transfer in the environment [23]. According to a study of Lay *et al.* [24], *bla* $_{\text{CTX-M-14}}$, a member of CTX-M Group 9 has been reported in *Escherichia coli* but not in *Salmonella*. In our study, only CTX-M Group 9 was detected, and no further subgroups were identified. To deepen understand of this possibly horizontal gene transfer mechanisms, further study to identify specific subgroup of CTX-M should be applied.

4. CONCLUSIONS

In conclusion, the primary sources of *Salmonella* contamination identified in this study include seafood, fish, meat and meat products with the presence of multidrug-resistant (MDR) and extended-spectrum β-lactamase (ESBL)-producing strains. Molecular screening revealed that nearly half of the ESBL-producing *Salmonella* isolates carried *bla* $_{\text{CTX-M}}$ group 9 genes, while *bla* $_{\text{CTX-M}}$ group 1, *bla* $_{\text{TEM}}$, or *bla* $_{\text{SHV}}$ genes were not detected. Despite confirmed phenotypic resistance, several isolates lacked detectable resistance genes, suggesting the potential presence of non-targeted ESBL genes. These findings underscore the genetic diversity of resistance mechanisms in *Salmonella* and the growing concern over antimicrobial resistance (AMR) in the food chain. The emergence of MDR and ESBL-producing *Salmonella* in food-producing animals highlights the urgent need for regulatory measures to restrict and monitor antibiotic use in agriculture and aquaculture. Antibiotic misuse and overuse in these sectors contribute significantly to treatment failures and persistent infections in humans. Therefore, strengthening AMR surveillance systems, improving food hygiene practices, and promoting responsible antibiotic use are critical. Further studies should employ other PCR primers to determine the remaining ESBL-producing strains in combination with sequencing to define the specific resistance genes, or whole genome sequencing, to comprehensively identify resistance genes and trace the spread of resistant Salmonella strains in the food production environment.

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REFERENCES

[1] U.S. Food and Drug Administration, "Get the facts about *Salmonella*," *FDA*, [Online]. Available: https://www.fda.gov/animal-veterinary/animal-health-literacy/get-facts-about-salmonella (accessed on 13 May 2024).

[2] T. M. Gomez, Y. Motarjemi, S. Miyagawa, F. K. Käferstein, and K. Stöhr, "Foodborne salmonellosis," *World Health Stat. Q.*, vol. 50, no. 1–2, pp. 81–89, 1997.

[3] B. Bouchrif *et al.*, "Prevalence and antibiotic-resistance of *Salmonella* isolated from food in Morocco," *J. Infect.*

Dev. Ctries., vol. 3, no. 1, pp. 35–40, 2009, doi: 10.3855/jidc.103.

[4] B. M. Marshall and S. B. Levy, "Food animals and antimicrobials: Impacts on human health," *Clin. Microbiol. Rev.*, vol. 24, no. 4, pp. 718–733, 2011, doi: 10.1128/CMR.00002-11.

[5] F. C. Cabello, "Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment," *Environ. Microbiol.*, vol. 8, no. 7, pp. 1137–1144, 2006, doi: 10.1111/j.1462-2920.2006.01054.x.

[6] World Health Organization, "Antimicrobial resistance," *WHO*, [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance (accessed on 13 May 2024).

[7] Y. Muktar, G. Abera, and J. Kemal, "Antibiotic resistance in *Salmonella* species, a serious public health problem: A review," *Global Veterinaria*, vol. 15, no. 5, pp. 469–479, 2015, doi: 10.5829/idosi.gv.2015.15.05.96225

[8] D. T. A. Nguyen *et al.*, "Prevalence, serovar, and antimicrobial resistance of nontyphoidal *Salmonella* in vegetable, fruit, and water samples in Ho Chi Minh City, Vietnam," *Foodborne Pathog. Dis.*, vol. 18, no. 5, pp. 354–363, 2021, doi: 10.1089/fpd.2020.2891.

[9] F. Medalla *et al.*, "Estimated incidence of antimicrobial drug–resistant nontyphoidal *Salmonella* infections, United States, 2004–2012," *Emerg. Infect. Dis.*, vol. 23, no. 1, pp. 29–37, 2017, doi: 10.3201/eid2301.160771.

[10] K. S. Lay, Y. Vuthy, P. Song, K. Phol, and J. L. Sarthou, "Prevalence, numbers and antimicrobial susceptibilities of *Salmonella* serovars and *Campylobacter* spp. in retail poultry in Phnom Penh, Cambodia," *J. Vet. Med. Sci.*, vol. 73, no. 3, pp. 325–329, 2011, doi: 10.1292/jvms.10-0373.

[11] M. Nadimpalli *et al.*, "CTX-M-55-type ESBL-producing *Salmonella enterica* are emerging among retail meats in Phnom Penh, Cambodia," *J. Antimicrob. Chemother.*, vol. 74, no. 2, pp. 342–348, 2019, doi: 10.1093/jac/dky451.

[12] EUCAST, "AST of bacteria," *European Committee on Antimicrobial Susceptibility Testing*, [Online]. Available: https://www.eucast.org/ast_of_bacteria (accessed on 06 May 2024).

[13] EUCAST, "Breakpoint tables for interpretation of MICs and zone diameters, Version 14.0," *European Committee on Antimicrobial Susceptibility Testing*, 2024. [Online]. Available: http://www.eucast.org (accessed on 06 May 2024).

[14] A-P. Magiorakos *et al.*, "Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance," *Clin. Microbiol. Infect.*, vol. 18, no. 3, pp. 268–281, 2012, doi: 10.1111/j.1469-0691.2011.03570.x.

[15] EUCAST, "Resistance mechanisms," *European Committee on Antimicrobial Susceptibility Testing*, [Online]. Available: https://www.eucast.org/resistance_mechanisms (accessed on 01 April 2025).

[16] L. Huoy *et al.*, "Prevalence of *Salmonella* spp. in meat, seafood, and leafy green vegetables from local markets and vegetable farms in Phnom Penh, Cambodia," *Food Microbiol.*, vol. 124, no. 104614, pp. 1–8, 2024, doi: 10.1016/j.fm.2024.104614.

[17] C. Marin *et al.*, "Research Note: Persistent *Salmonella* problems in slaughterhouses related to clones linked to poultry companies," *Poultry Science*, vol. 101, no. 101968, pp. 1–6, 2022, doi: 10.1016/j.psj.2022.101968.

[18] K. Chonsin *et al.*, "Prevalence and multidrug resistance of *Salmonella* in swine production chain in a central province, Thailand," *J. Food Prot.*, vol. 84, no. 12, pp. 2174–2184, 2021, doi: 10.4315/JFP-21-003.

[19] C. Om and M.-L. McLaws, "Antibiotics: Practice and opinions of Cambodian commercial farmers, animal feed retailers and veterinarians," *Antimicrob. Resist. Infect. Control*, vol. 5, no. 42, pp. 2–8, 2016, doi: 10.1186/s13756-016-0147-y.

[20] L. J. R. Salvador *et al.*, "Prevalence of multidrug-resistant *Salmonella* spp. in Asia: A mini-review," *Asian J. Biol. Life Sci.*, vol. 11, no. 2, pp. 267–275, 2022, doi: 10.5530/ajbls.2022.11.36.

[21] Z. Chen *et al.*, "Highly prevalent multidrug resistance and QRDR mutations in *Salmonella* isolated from chicken, pork and duck meat in Southern China, 2018–2019," *Int. J. Food Microbiol.*, vol. 340, no. 109055, pp. 1–7, 2021, doi: 10.1016/j.ijfoodmicro.2021.109055.

[22] É. Ruppé, P.-L. Woerther, and F. Barbier, "Mechanisms of antimicrobial resistance in Gram-negative bacilli," *Ann. Intensive Care*, vol. 5, no. 21, pp. 2–15, 2015, doi: 10.1186/s13613-015-0061-0.

[23] C. O. Fakorede, K. O. Amisu, M. Saki, and K. O. Akinyemi, "Co-existence of extended-spectrum β-lactamases *bla*CTX-M-9 and *bla*CTX-M-15 genes in *Salmonella* species isolated from febrile and diarrhoeagenic patients in Lagos, Nigeria: A cross-sectional study," *Eur. J. Med. Res.*, vol. 28, no. 3, pp. 1–15, 2023, doi: 10.1186/s40001-022-00960-0.

[24] K. K. Lay, S. Jeamsripong, K. P. Sunn, S. Angkititrakul, R. Prathan, S. Srisanga, and R. Chuanchuen "Colistin resistance and ESBL production in *Salmonella* and *Escherichia coli* from pigs and pork in the Thailand, Cambodia, Lao PDR, and Myanmar border area," *Antibiotics*, vol. 10, no. 6, pp. 657–669, 2021, doi: 10.3390/antibiotics10060657.