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Occurrence and characteristics of Salmonella isolated from various vegetable sources: Potential for the human-food interface in salmonellosis in Vientiane, the capital of Laos PDR

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Occurrence and characteristics of *Salmonella* isolated from various vegetable sources: Potential for the human-food interface in salmonellosis in Vientiane, the capital of Laos PDR

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Abstract

Salmonella is one of the most frequently reported causes of bacterial foodborne outbreaks in the world. Despite this, cases associated with raw vegetable consumption have increased, however very few studies have focused on this. The present study is an overview of the role of vegetables as a concern for *Salmonella* circulating in Vientiane, the capital of the Lao People's Democratic Republic (Lao PDR). A cross-sectional study was surveyed during the first half 2019 on various types of vegetable sold in retailers in the area. The prevalence, serotype, antimicrobial resistance pattern and genetic profile of *Salmonella* were investigated. In this study, a total of 80 vegetable samples were collected. 26 (32.5%) positive isolates consisting of 13 different serotypes were determined. *S. Corvallis* was the most common serotype detected (4/26; 15.4%). Six (23.1%) isolates were found to be resistant to ampicillin, and five (19.2%) isolates resisted sulfamethoxazole-trimethoprim, tetracycline and amoxicillin-clavulanic acid. In addition, a genetic comparison of the vegetable-originated *Salmonella* with the isolates recovered from hospitalized patients and meats for consumption was carried out using Multilocus sequence typing (MLST). Identical clones of "ST469-S. Rissen" derived from humans, meat and vegetables were evidenced. The inter-species transmission between hosts highlights an alarming possible trend of human-food interface in salmonellosis in Vientiane. Thus, educating individuals on hygienic practices should be recommended. Likewise, intersectoral collaborations should be paid more attention together with scale-up intervention to the entire food chain at the policy level for successful salmonellosis control.

Keywords: Human-food interface, Lao PDR, Multilocus Sequence Typing, *Salmonella*, vegetable

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Introduction

Foodborne illness associated with *Salmonella* continues to pose a serious health threat of zoonotic diarrheic disease outbreak in the world (Coker *et al.*, 2011; Lawpoolsri *et al.*, 2018; Wiedemann *et al.*, 2015; Zellweger *et al.*, 2017). Commonly, the infection is acquired through consumption of contaminated animal-origin foods (Majowicz *et al.*, 2010). Domestic animals including pigs, cattle and poultry are often considered as the primary reservoir of *Salmonella* (Mughini-Gras *et al.*, 2014). The pathogen usually multiplies in an animal's guts and spreads horizontally between animals, via the fecal-oral route directly or through the environment at farm level (Palmer and Slauch, 2017). Inadequate hygienic practices at the further level, slaughtering, processing, chilling or cooking procedures can increase the contamination pressure downstream for end consumers (Akil and Ahmad, 2019; Patchanee *et al.*, 2016). Besides, via contaminated feces, *Salmonella* can also enter the agricultural community. Contamination of plant sources or vegetables is possible through the use of organic fertilizers and crop irrigation with wastewater (Quiroz-Santiago *et al.*, 2009). Anyway, improper storage conditions or any unhygienic practices in the purchasing area can play a role as a motivating factor of re-contamination. Numbers of food poisonings related to contaminated raw vegetables have been increasing (Berger *et al.*, 2010; Heaton and Jones, 2008; Quiroz-Santiago *et al.*, 2009), however very few studies have focused on this issue. Therefore, this is the opportunity to fill this knowledge gap.

In Southeast Asia, many cases with many deaths due to foodborne illness have been continually reported (Coker *et al.*, 2011; Zellweger *et al.*, 2017), especially in developing areas such as the Lao People's Democratic Republic (Lao PDR) (Lawpoolsri *et al.*, 2018), even though, in the country, Gross Domestic Product (GDP) and infrastructure have been improving for over a decade (Midorikawa *et al.*, 2016). Nevertheless, this has not enhanced the public health management system to a sufficient level, even in the capital city of Vientiane. Farming practices are predominantly traditional and applied without Good Agricultural Practice (GAP) standards. Moreover, at the post-harvesting level, uncontrolled storage conditions in purchasing areas at the retailers have been commonly found. (Meunsene *et al.*, 2021). Focusing on *Salmonella* in the city, many studies have shown that contamination may occur at all levels of the food chain (Boonmar *et al.*, 2013; Inthavong *et al.*, 2006; Sinwat *et al.*, 2016; Somsanith *et al.*, 2012), also in human (Le Thi Phuong *et al.*, 2017; Midorikawa *et al.*, 2016). *Salmonella* circulating in the study area contains competence in antimicrobial resistance and seems to have the potential to cause disease in humans. However, an association of human salmonellosis and food products sold at retailers in the area has not been proven by scientific information or any molecular evidence, until now.

Recently, Meunsene *et al.*, (2021) reported high incidences of *Salmonella* isolated from meat for consumption (pork, chicken and beef) sold in Vientiane. Multi Locus Sequence Typing (MLST)

evidenced a diverse range of *Salmonella* genotypes which were distributed over the area. Likewise, the persistence of the residential flora and sharing of the contaminated supply route (farm-slaughterhouse-retail) within the country can be inferred. This work is the first comprehensive study of the transmission dynamics of *Salmonella* in the area and shows that MLST analysis can provide crucial information for epidemiological study. The technique is most suitable for the study of genetic comparison of bacterial strains recovered from different sources in the various distributions (Mirajkar and Gebhart, 2014; Zhou *et al.*, 2020). As MLST is based on nucleotide sequences analysis of housekeeping genes, so the results from different laboratories can easily be compared (Liu *et al.*, 2011).

In response to current concerns, the aims of the study were: (1) to determine the prevalence of *Salmonella* contamination in various vegetable sources sold in retailers in Vientiane the capital of Lao PDR. (2) to explain the phenotypic characteristics (antimicrobial resistance patterns and serotypes) of isolated *Salmonella* and (3) to define an overview of the role of human-food interface in salmonellosis in the study area. Acquiring information will inform us of the *Salmonella* transmission dynamics and provide the basis for the development and implementation of salmonellosis controls in the region.

Materials and Methods

Samples collection: Fresh vegetables were collected from 5 local retailers in Vientiane, Lao PDR from January to May 2019. The sample size of this study was determined using the Win Epi online program (<http://www.winepi.net/uk/index.htm>). A *Salmonella* prevalence of 5.2% from a study in Thailand (Ananchaipattana *et al.*, 2012) was chosen as the % expected prevalence. An accepted error of 5% and confidence levels of 95% with an infinite population size were selected for the required features input. From the calculation procedure, at least 76 samples were required. Definitely, 80 fresh vegetable samples were carefully collected.

Samples were obtained from the vegetables that Lao people prefer eating raw, which were easy to find in any retailers. Nineteen different vegetables were collected, including basil (*Ocimum basilicum*) (n=5), bean sprouts (*Vigna radiata*) (n=3), celery (*Apium graveolens*) (n=5), chili (*Capsicum annum*) (n=5), coriander (*Coriandrum sativum*) (n=7), cilantro (*Eryngium foetidum*) (n=1), dill (*Anethum graveolens*) (n=5), eggplant (*Solanum virginianum*) (n=5), gotu kola (*Centella asiatica*) (n=5), green onion (*Alliumcepa aggregatum*) (n=5), houttuynia (*Houttuynia cordata*) (n=3), lettuce (*Lactuca sativa*) (n=5), long bean (*Vigna sesquipedalis*) (n=5), mustard greens (*Brassica juncea*) (n=1), peppermint (*Mentha piperita*) (n=5), tomato (*Lycopersicon esculentum*) (n=5), water convolvulus (*Ipomoea aquatica*) (n=5), water spinach (*Ipomoea aquatica*) (n=4) and yellow bur head (*Limocharis flava*) (n=1). Each sample was separately tagged, put into a sterile polyethylene bag and stored in an icebox for laboratory analysis within 24 hr at the Department of

Veterinary Medicine, Faculty of Agriculture, National University of Laos, Nabong Campus, Lao PDR.

Salmonella isolation and identification: Isolation and identification of *Salmonella* spp. from fresh vegetables were performed following ISO6579: 2002 Amendment 1:2007, Annex D technique. Twenty-five g of vegetable samples were added with 225 ml of Buffered Peptone Water (BPW; Merck, Germany). The mixing materials were homogenized for 120 secs and incubated at 37°C for 24 hrs. An aliquot of 100 µl of the pre-enriched inoculum was transferred to 10 ml tubes with Rappaport-Vassiliadis broth (RV; Oxoid, UK). All materials were mixed carefully, and were incubated at 42°C for a period of 24 hrs. The presumptive colonies were then streaked on Xylose-Lysine-Desocholate agar (XLD; Oxoid, UK) and Brilliant-green Phenol Red Lactose sucrose agar (BPLS; Merck, Germany). After an incubation period of 24 hrs at 37°C, the selective colonies, black in color, were then subjected to Triple Sugar Iron agar (TSI; Oxoid, England), urease and Motility Indole-Lysine agar (MIL; Merck, Germany) for bio-chemical identification. All positive results were then marked. Overall *Salmonella* incidence and the value separated in each vegetable type with their 95% confidence level were determined by descriptive statistical analysis using Epi Info™ version 7 (Centers for Disease Control and Prevention, Georgia, United States).

Serotyping and antimicrobial susceptibility testing:

An individual *Salmonella* isolate (only one isolate per one positive sample) was serotyped according to the White-Kauffmann-Le Minor scheme (Popoff et al., 1993). All positive isolates were tested for susceptibility to amoxicillin-clavulanic acid (AMC) 20/10 µg, ampicillin (AMP) 10 µg, chloramphenicol (C) 30 µg, ciprofloxacin (CIP) 5 µg, cefotaxime (CTX) 30 µg, nalidixic acid (NA) 30 µg, norfloxacin (NOR) 10 µg, streptomycin (S) 10 µg, sulfamethoxazole-trimethoprim (SXT) 23.75/1.25 µg, and tetracycline (TE) 30 µg on Mueller-Hinton agar plates by the disc diffusion method (CLSI, 2011). Isolates that resisted three or more of the antimicrobial agents were considered in multidrug resistance. Inter-relationship between the categories of vegetable source, serotype and antimicrobial resistant pattern were visualized in chord diagrams using Power BI (Microsoft, Washington, United States).

Multi-locus Sequence Typing (MLST): Randomly selected 3 *Salmonella* isolates taken from the most majority serotypes were genotyped using the MLST technique. DNA was extracted according to the protocol described by Liu et al., (2011). PCR amplification of seven housekeeping genes, including *aroC* (chorismate synthase); *dnaN* (DNA polymerase III beta subunit); *hemD* (uroporphyrinogen III cosynthase); *purE* (phosphoribosylaminoimidazole carboxylase); *sucA* (alpha ketoglutarate dehydrogenase); *hisD* (histidinol dehydrogenase) and *thrA* (aspartokinase I/homoserine dehydrogenase), were accomplished using the method previously conducted by Meunsene et al., (2021). The PCR products were sequenced at the MacroGen Service Center, Republic of Korea. To get the

Sequence Type (ST), sets of 7 allelic numbers from each isolate (Table S1) were filled in the *Salmonella enterica* MLST Database, Warwick medical school, The University of Warwick <http://mlst.warwick.ac.uk/mlst/dbs/Senterica>).

Minimum spanning trees were generated using Bionumerics® software version 7.2 (Applied Maths, Sint-Martens Latem, Belgium). Three isolates acquired during the current study, 25 *Salmonella* isolates of Meunsene et al., (2021) study, which were recovered from meats sold in Vientiane capital (8, 12 and 5 isolates from pork, beef and chicken, respectively) and an additional of 12 *Salmonella* isolated from hospitalized patients who were admitted to Mahosot Hospital in Vientiane, Lao PDR were compiled for an analysis.

Regarding the 12 human isolates acquired, two hundred and fifty stool samples were initially collected during January to June 2019. All of those were selected from patients who had a history of diarrhea. Each of the individual isolates were serotyped and tested for antimicrobial susceptibility, as well MLST genotyped (Table 1) at the Department of Veterinary Medicine, Faculty of Agriculture, National University of Laos, Nabong Campus, Lao PDR (with the same procedures of vegetable isolates). Research protocols for human subjects were approved by Ministry of Health, Lao PDR, National Ethics Committee for Health Research (NECHR) (Submission ID: 2018.113.Vie) in No. 07/NECHR, Vientiane 17/01/2019.

Results

Salmonella prevalence: From 80 vegetable samples collected during January to May 2019, *Salmonella* was detected in 32.5% (26/80; 95% CI= 22.45-43.89%). Eleven out of 19 different types of fresh vegetables were positive with a range of 20-80%. Peppermint demonstrated the highest contamination rate (80%; 4/5), followed by bean sprouts (66.67%; 2/3) and basil, lettuce and water convolvulus (60%; 3/5), respectively. Nevertheless, no positive samples could be detected from cilantro, dill, eggplant, green onion, houuttuynia, mustard greens, tomato and yellow bur head (Table 2).

Salmonella serotyping and antimicrobial susceptibility testing: twenty-two of all 26 isolates belonged to 13 serotypes, while the 4 remaining were considered untypable. A chord diagram visualizing the vegetable source-serotype relationships is displayed in Fig. 1A. Overall, there was no relationship between those two categories considered, with the exception of two isolates recovered from long bean. Those were *S. Corvallis*, which was the most common serotype detected (15.38%; 4/26). Just as mentioned before, *S. Corvallis* was distributed in two of the long bean samples and for each from basil and water convolvulus. *S. Brunei* and *S. Stanley* were placed as followers (11.54%; 3/26). One each of basil, bean sprout and chili were detected as *S. Brunei* positive. For another serotype, *S. Stanley* was distributed in a sample of basil, celery and coriander. Interestingly, one of typhoidal *Salmonella* was also detected. The *S. Paratyphi* was isolated from a peppermint sample.

Fig. 1B displays serotype-antimicrobial resistance relationships of the 26 *Salmonella* isolates. More than a half (53.85%; 14/26) were susceptible to all ten antimicrobials investigated. Within these, all four *S. Corvallis* detected were remarkably comprised in this pan susceptible group. Other than that, there was no distinctive pattern of antimicrobial resistance that was serotype specific. The resistance of all isolates tested was highest for ampicillin (23.07%; 6/26), followed by

sulfamethoxazole-trimethoprim, tetracycline and amoxicillin-clavulanic acid (19.23%; 5/26). None of the isolates showed resistance against enrofloxacin and ciprofloxacin. Five isolates (19.23%) were classified as multidrug resistant. Four of those resisted against five antimicrobials tested including AMP/AMC/S/SXT/TE (*S. Agona* and *S. Stanley*), AMP/C/S/SXT/TE (*S. London*) and AMP/AMC/C/SXT/TE (untypeable).

Table 1 Origin and characterisation of *Salmonella* spp. isolated from hospitalized patients at Mahosot Hospital in Vientiane, Lao PDR, during January to June 2019.

Sample ID	Admission date	Address location ^a	Serotype	ABO- resistance pattern ^b	ST-type ^c	ST-complex
HL01	3-Jan-2019	Hadxayfong	<i>S. Rissen</i>	AMP/C/CTX/NA/S/SXT/TE	-	66
HL02	8-Feb-2019	Sisattanak	<i>S. Stanley</i>	all susceptible	ST29	29
HL04	15-Mar-2019	Sikhottabong	<i>S. Uganda</i>	AMP/C/CTX/S/TE	ST684	157
HL05	21-Mar-2019	Xaythany	<i>S. Typhimurium</i>	AMP/C/SXT/TE	ST19	1
HL06	22-Mar-2019	Sikhottabong	<i>S. Rissen</i>	AMP/AMC/C/CTX/NA/S/SXT/TE	ST469	66
HL07	29-Mar-2019	Sikhottabong	<i>S. Newport</i>	all susceptible	-	57
HL08	3-Apr-2019	Xaythany	<i>S. Weltevreden</i>	AMP/AMC/C/CTX/NA/SXT/TE	-	3
HL09	20-Apr-2019	Sikhottabong	<i>S. Rissen</i>	AMP/AMC	ST469	66
HL10	18-Apr-2019	Xaythany	<i>S. Hvitittingfoss</i>	AMP/AMC/C/SXT/TE	-	259
HL11	23-May-2019	Chuanthabuly	<i>S. Bareilly</i>	AMP/AMC/CTX/NA/S/SXT/TE	ST203	206
HL13	29-May-2019	Sikhottabong	<i>S. Kedougou</i>	AMP/AMC/S/SXT/TE	-	291
HL14	25-Jun-2019	Xaythany	<i>S. Agona</i>	AMP/AMC/C/S/SXT/TE	ST3634	54

^aThe information in this column indicates the district (of Vientiane) where the patient lives.

^bAbbreviations in the column: ampicillin (AMP); amoxicillin-clavulanic acid (AMC); chloramphenicol (C); cefotaxime (CTX); nalidixic acid (NA); streptomycin (S); sulphamethoxazole-trimethoprim (SXT); tetracycline (TE).

^cThe blank space in this column indicates the isolate could not be assigned to any known ST.

Table 2 Incidence of *Salmonella* spp. distributed in vegetables sold in fresh markets in Vientiane, Lao PDR, during January to May 2019.

Vegetables	Numbers collected	Numbers positive	% Positive	95% Confidence interval
Basil	5	3	60.00	14.66-94.73
Bean sprouts	3	2	66.67	9.43-99.16
Celery	5	1	20.00	0.51-71.64
Chili	5	1	20.00	0.51-71.64
Coriander	7	3	42.86	9.90-81.59
Culantro	1	0	0.00	0.00-97.5
Dill	5	0	0.00	0.00-52.18
Eggplant	5	0	0.00	0.00-52.18
Gotu kola	5	2	40.00	5.27-85.34
Green onion	5	0	0.00	0.00-52.18
Houttuynia	3	0	0.00	0.00-70.76
Lettuce	5	3	60.00	14.66-94.73
Long bean	5	2	40.00	5.27-85.34
Mustard greens	1	0	0.00	0.00-97.5
Peppermint	5	4	80.00	28.36-99.49
Tomato	5	0	0.00	0.00-52.18
Water convolvulus	5	3	60.00	14.66-94.73
Water spinach	4	2	50.00	6.76-93.24
Yellow bur head	1	0	0.00	0.00-97.5
TOTAL	80	26	32.50	22.45-43.89

Minimum spanning tree (MST): Random selections of 3 vegetable-origin isolates were taken to get the ST-type (Table 3). To expand epidemiological knowledge of the human-food interface in salmonellosis in Vientiane, MST analysis of those 3 isolates, 12 human isolates and 25 meats-origin isolates from Meunsene *et al.*, (2021) was conducted (Fig. 2). Of all 40 isolates analyzed, 34 ST profiles of 5-ST complex clusters demonstrated 5 different host origins. In overview, more than three

quarters of all Vientiane's isolates were unique to a single host origin, with the exception of the isolates grouped in ST469, ST155 and ST365. ST469 was the most frequently noticed. Members grouped in the ST were recovered from hospitalized patients, beef, pork and fresh vegetables. Of the ST155 members, one was isolated from pork and another was from a chicken sample. Likewise, each isolate originating from beef and chicken samples belonged to ST365.

Table 3 Origin and characterisation of *Salmonella* spp. isolated from vegetable sources at retailers in Vientiane, Lao PDR.

Sample ID	Sampling date	Vegetable type	Retail ^a	Serotype	ABO- resistance pattern ^b	ST-type ^c	ST-complex
LVE17	20-May-2019	Water convolvulus	Phonsavang	S. London	CTX	-	252
LVE18	20-May-2019	Lettuce	Phonsavang	S. Rissen	AMP/C/SXT/TE	ST469	66
LVE24	28-May-2019	Basil	Thongkhankham	S. Corvallis	all susceptible	-	236

^aThe information in this column indicates the retailer's name where the sample was taken.

^bAbbreviations in the column: ampicillin (AMP); chloramphenicol (C); cefotaxime (CTX); sulphamethoxazole-trimethoprim (SXT); tetracycline (TE).

^cThe blank space in this column indicates the isolate could not be assigned to any known ST.

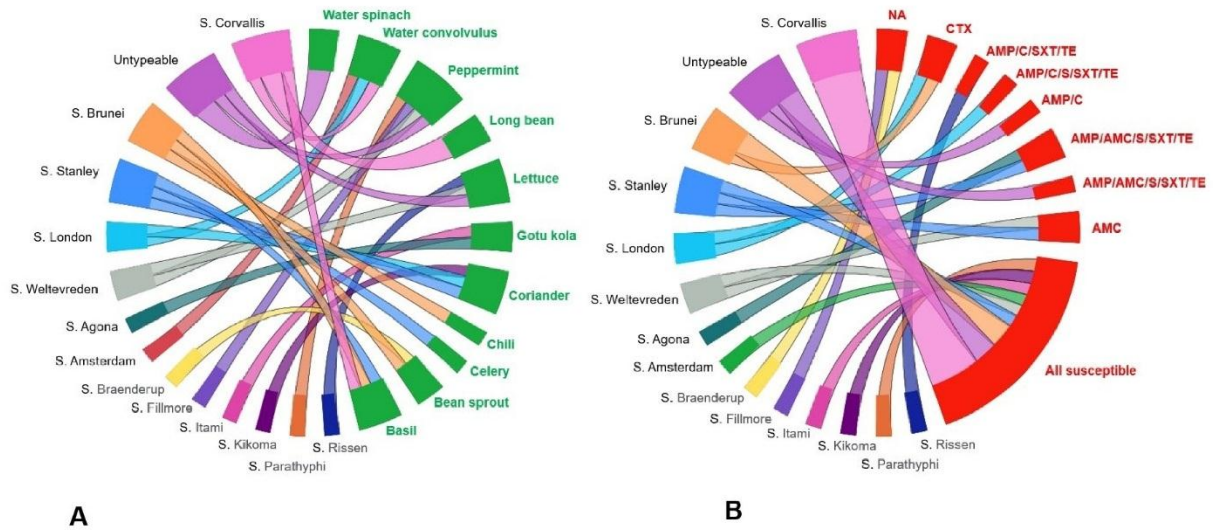


Figure 1 Chord diagram analysis of characteristics with their origin of *Salmonella* spp. isolated from vegetables sold in Vientiane, during January to May 2019.

A: association of *Salmonella* serotype with their origin isolated

B: association of *Salmonella* serotype with their antimicrobial resistant pattern

Antibiotic abbreviation: amoxicillin-clavulanic acid (AMC); ampicillin (AMP); chloramphenicol (C); cefotaxime (CTX); nalidixic acid (NA); sulfamethoxazole-Trimethoprim (SXT); streptomycin (S); tetracycline (TE).

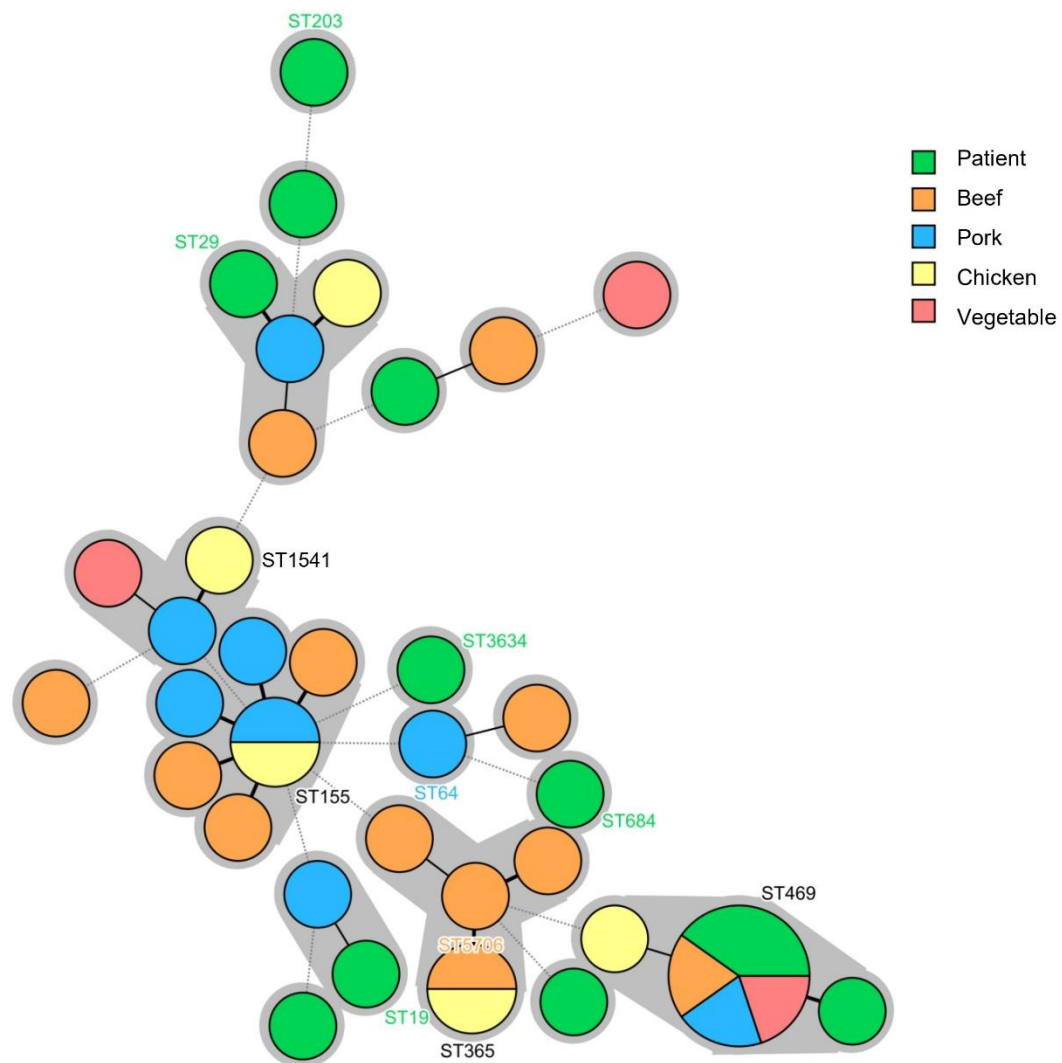


Figure 2 Minimum spanning tree analysis of 40 *Salmonella* strains collected from pork, beef, chicken, vegetable and hospitalized patients in Vientiane, Lao PDR. Each circle represents a different ST profile. The size of each circle displays the number of isolates and the color indicates the host of origin. Black thick solid lines connect ST-types with one locus difference; black thin solid lines connect ST-types with double and three loci differences; grey dashed lines connect four loci differences; grey dotted lines connect more than four loci differences, respectively. Grey shaded areas represent at least five related loci between STs (grouping in common ST-complex).

Discussion

Vegetables are full of essential dietary fiber, minerals, vitamins and antioxidants that provide health benefits for the body's metabolic function. Since they are of low energy density with the little fat content, dietary guidelines recommend daily intake (Herforth *et al.*, 2019). However, due to the relatively high pH with high surface/weight ratio, vegetables can host a large bacterial population (Pezzuto *et al.*, 2016). In this study, the presence of 32.5% (26/80; 95% CI= 22.45-43.89%) prevalence of *Salmonella* indicated the high risk of vegetable consumption in Vientiane, Lao PDR. This result is not close to previously studies which were conducted in nearby areas. In the Southeast Asia region, prevalence of 5.2% (2/38; 95% CI= 0.64-17.75%) and 12.9% (74/572; 95% CI= 10.43-15.94%) of *Salmonella*-contaminated vegetables was

reported in retailers in Thailand (Ananchaipattana *et al.*, 2012) and Vietnam (Nguyen *et al.*, 2021), respectively. In Shanghai, China, Ni *et al.*, (2018) informed that a 4.16% (2/46; 95% CI= 0.51-14.25%) positive rate of *Salmonella* isolated from vegetable was sold in wet markets. Sanitation practice is a key factor related to the occurrence of such outcomes. Standard protocols for the retailers in our study area tended to be low. In Laos PDR, agricultural activity often lacks sufficient facilities and investment (Midorikawa *et al.*, 2016). Hygiene during growth, harvest, storage as well as distribution does not receive adequate attention. Raw canal or any wastewater is often used in crop irrigating. Likewise, contact with contaminated harvesting equipment such as knives, workers' hands or gloves and containers is also of concern as an additional related factor. Especially through the cut edges in the process of core removal, vegetable tissues

are damaged and further human manipulating can possibly increase microbial infiltration (Jung *et al.*, 2014). Moreover, at the retail purchasing area, under the relatively high temperature of the environment, vegetables are often placed directly on benches or the floor without covering materials.

Salmonella contamination is varied among vegetable types. Peppermint was detected as the highest frequently found in *Salmonella*. Then, eight vegetables; cilantro, dill, eggplant, green onion, houttuynia, mustard greens, tomato and yellow bur head could not be detected. Based on the study purpose, all 80 samples utilized were not designed for comparing the prevalence between vegetable types. Since the 95% confidence interval of each was demonstrated in a wide range, the observed incidence in that sub-populations may not be representative of each type of vegetable being sold in other areas.

In our work, thirteen typeable serotypes were identified. *S. Corvallis* was the most frequently isolated. This corresponds with the recent study of Meunsene *et al.*, (2021). This serotype was ranked in the top-two serotypes detected in meat for consumption in the Vientiane area. The situation is quite similar with the others serotype. *S. Agona*, *S. Amsterdam*, *S. Brunei*, *S. Itami*, *S. London*, *S. Rissen*, *S. Stanley* and *S. Weltevreden* were also demonstrated as matching serotypes detected in both animal and vegetable sources circulating in the study area. A sharing pool of *Salmonella* seems to be inferred. However, in the newly reported serotype in this country, *S. Baenderup*, *S. Fillmore* and *S. Kikoma* were noticed. To the best of our knowledge, we are therefore unable to deduce any prior source of contamination. It is a very interesting issue that should be addressed in further. Well, one occurrence of typhoidal *Salmonella* was found, *S. Paratyphi*. It is one of the major issues in global health concerns and highlights a potential that has been causing an epidemic disease in humans, called enteric fever. Without prompt treatment, it can result in serious complications and can be fatal (Thandassery *et al.*, 2014). Anyway, four isolates were not typeable in our laboratory. There are two reasons to support this. The occurrence of somatic antigens synthesis alterations can make it self-agglutinable, and inability to detect the flagella phase might happen (Quiroz-Santiago *et al.*, 2009).

In the current study, more than a half of *Salmonella* were susceptible to all ten antimicrobials investigated. That contrasts with the studies of Sinwat *et al.*, (2016) and Meunsene *et al.*, (2021) conducted in the same area where pan-susceptible isolates have been usually reported in a relatively low number. However, many *Salmonella* spp. isolated from slaughtered buffalo from Boonmar *et al.*, (2013) were also demonstrated to be pan susceptible. The finding in our study is quite complicated and difficult to validate. Even so, we can prove that using of antimicrobials in an animal farming agricultural system can provide an effect in selective pressure on animal guts and can cause a cross-contamination effect to the cultivated area via manure and also wastewater.

Typically, salmonellosis in humans takes the form of a self-limiting, however, in some severe systemic progressions, antimicrobials should be administered

(Sodagari *et al.*, 2020). High resistance rates of ampicillin, sulfamethoxazole-trimethoprim and tetracycline have been demonstrated for a half decade in this region (Meunsene *et al.*, 2021; Patchanee *et al.*, 2016; Tadee *et al.*, 2015; Yang *et al.*, 2019). It is confirmed that those antimicrobial agents have not been effective in salmonellosis treatment. Surprisingly, a high rate of resistance against amoxicillin-clavulanic acid has been recorded at this time. Even though, this agent is one of the most frequently used broad-spectrum antimicrobials in emergency departments and primary care offices. It has activity against Gram-positive and Gram-negative bacteria, including *Streptococcus* spp., *Enterococcus* spp., *Neisseria* spp., *Proteus* spp., *Staphylococcus aureus*, *Escherichia coli*, as well as *Salmonella* (Evans *et al.*, 2021). But then, from the findings at this moment, it is not approved for salmonellosis treatment in our study area. Nevertheless, with 0% resistance of ciprofloxacin and enrofloxacin, fluoroquinolones are still required for the first line drug use for invasive *Salmonella* (Effa *et al.*, 2011; Thai *et al.*, 2021).

MST analysis provided the genetic population structure of 40 *Salmonella* circulating in Vientiane during the first half of 2019. Thirty-four ST profiles of 5 ST-complex clusters, with many untypable ST were investigated. Microevolution or mutation of geographical isolates is a possible explanation for these genetically diversities (Liu *et al.*, 2011). In addition, shared ST profiles of the local isolates (ST155, ST365 and ST479) suggest the likelihood of a common infection source (Mirajkar and Gebhart, 2014). Tracing to the origins of isolates grouping in ST155 and ST365, the clonal groups were obtained from identical places with different sampling dates. The persistent or residential *Salmonella* were evidenced as described by Meunsene *et al.*, (2021). Underlining, isolates grouped in ST469 belonged to multiple host origins, human-meats-vegetable. It can be proved that "ST469-S. Rissen" can maintain their genetic identity across agricultural-livestock community likely caused by anthropogenic environmental contamination and this has the potential for spillover entry into the human food chain in the study area.

Salmonella infection outbreaks associated with vegetable consumption are often overlooked. Consumers are frequently unaware of their role and ignore adequate practices in sanitation and eating habits. This study indicates that vegetables are one of the primary *Salmonella* reservoirs, which can cause the disease in humans and enter the food chain along the stages of "from farm to plate". Multi-locus Sequence Typing (MLST) demonstrates the genetic linkage of *Salmonella* obtained from multi host origins. It underlines the possible noticeable evidence of human-food interface in that area. The acquisition of knowledge gathered in this study makes it clear that attention must be given to entries in the food chain to successfully control possible contamination at all levels. Formal intervention by the regional multidisciplinary health authorities is also necessary. Lastly, the provision of education related to good personal hygiene practices may significantly mitigate risks of foodborne disease.

Conflict of Interest: We declare that we have no conflict of interest.

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