Original Article

Seasonal and antimicrobial resistance distributions of *Salmonella* isolated from pork, beef and chicken meat in Vientiane Capital, Lao PDR

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Abstract

The objectives in this study was to investigate the prevalence, seasonal variation and antimicrobial resistance of *Salmonella* isolated from pork, beef and chicken meat at retail markets in Vientiane capital, Lao PDR. During May 2018 to April 2019, 445 swab samples were randomly collected from pork (n = 258), beef (n = 81) and poultry meat (n = 106) in three seasons at three retail big markets in Vientiane capital, Lao PDR, Thongkhankham, Lao-Aussi, and Nongchan markets. All samples were isolated and identified for *Salmonella* spp. using the ISO 6579:2002/AMD 1:2007 procedure. The isolates were tested for antimicrobial susceptibility using the Kirby-Bauer method. The prevalence of *Salmonella* contamination isolated from pork, beef and chicken meat at retail markets in Vientiane capital, Lao PDR were 49.2%, 61.7% and 61.3% respectively. Significantly higher number of *Salmonella* spp. was observed in beef and chicken meat during rainy season. The most common serotype in pork, beef and chicken meat was *S.* Rissen (37.0%), *S.* Derby (10.8%) and *S.* Corvallis (38.0%), respectively. The highest resistance was found for TET (72.3%), followed by AMP (62%), SXT (51.7%) and CHL (26%). Hygiene and sanitation of slaughterhouse and retail markets should be concerned to control and prevent of *Salmonella* spp. contamination along the food supply chain.

Keywords: Seasonal, Antimicrobial Resistance, *Salmonella,* Lao PDR

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Received July 5, 2021

Accepted October 15, 2021

https://doi.org/10.14456/tjvm.2022.6

Introduction

Salmonellosis, a major food-borne zoonotic disease, caused by *Salmonella* spp. in humans and animals is an important public health problem worldwide. *Salmonella* spp. is one of the most important causes of human bacterial diarrheal infections worldwide. According to recent global estimates, 93.8 million cases of non-typhoidal *Salmonella* gastroenteritis occur annually, producing up to 155,000 deaths (Campos *et al*., 2019). In Southeast Asia, it is estimated that 22.8 million cases with 37,600 deaths occurred each year (Van *et al*., 2012). Cross-contamination during processing and inadequate sanitation in slaughterhouse together with the consumption of undercooked meat could serve as the most frequent causes of infection by *Salmonella* reported in humans (Carrasco *et al*., 2012). Contamination with *Salmonella* spp. may occur not only during the slaughtering process but also in the retail market (Vidayanti *et al*., 2021). Retail markets are popular places where food can be purchased at affordable prices, but they are often linked to outbreaks of disease due to their unhygienic conditions. Retail markets have been identified as the most significant point of contact for salmonellosis exposure and transmission among humans (Hauser *et al*., 2011; Gomes-Neves *et al*., 2012).

Currently, this public health concern has become complicated because of the rapid emergence and spread of *Salmonella* strains resistant to clinically important antimicrobial agents (Majowicz *et al*., 2010). Antimicrobial resistance (AMR) is evidently a result of over- and misuse of antimicrobials both in human and animal (WHO, 2020). Antimicrobials are extensively used in food-producing animal either treatment, prevention or promote growth (Van Boeckel *et al*., 2015; Coyne *et al*., 2019). For AMR in *Salmonella*, a strong association between resistant bacteria from human cases and those from food-producing animals was demonstrated (EFSA, 2017). AMR *Salmonella* isolate was commonly found in pigs, pork and human samples in Vientiane province (Sinwat *et al*., 2016). Diverse range of *Salmonella* genotypes were also observed spread over the area (Meunsene *et al*., 2021).

Vientiane is the capital and largest city of Laos. The city had a population of 948,477 peoples, covering an area of 3,920 square kilometers (Britannica.com, 2021). High amount of animal product such as pork, beef and chicken meat are consumed every day. Theorectically, the seasonal trend of increasing human salmonellosis in summer is clear (EFSA, 2009; 2011; 2012). However, few data of the seasonal distribution of meat *Salmonella* serotypes has been reported. The relatively high *Salmonella* spp. contamination rates of raw chicken meat and liver in summer have been detected in Greece (Zdragas *et al*., 2012).

The aim of this study was to investigate the prevalence, seasonal variation and antimicrobial resistance of *Salmonella* isolated from pork, beef and chicken meat at retail markets in Vientiane capital, Lao PDR.

Materials and Methods

Ethical approval: The study was approved by the Institutional Animal Care and Use Committee of Khon Kaen University (No. IACUC-KKU-27/63).

Sample collection: The sample size was calculated using the Win Epi online program (http://www.winepi.net/uk/index.htm). Percentage of expected prevalence from the previous studies of 25% (Somsanith *et al*., 2012), 82% (Boonmar *et al*., 2013) and 75% (Angkititrakul *et al*., 2005) for pork, beef and chicken meat, respectively, were used. An accepted error rate of 8.5% and 95% confidence levels were selected for the required inputs. Total of 100, 79 and 100 samples were obtained as the minimal needed sample size for pork, beef and chicken meat, respectively. From this minimal sample sized in each meat type, number of samples was planned to collect equally in 3 different seasons.

During May 2018 to April 2019, 445 swab samples were collected randomly from pork (n = 258), beef (n = 81) and poultry meat (n = 106) at top three big retail markets (high number of meat sell stall) in Vientiane capital, Lao PDR, Thongkhankham, Lao-Aussi, and Nongchan markets. Samples were collected in 3 seasons including winter season (November to February, 59-68 % humidity rate, 17-30 ℃ temperature; n = 185), summer (March to May, 58-77 % humidity rate, 22-34 ℃ temperature; n = 138) and rainy (June to October, 83-86 % humidity rate, 24-30 ℃ temperature; n = 122). All sample swabs were placed in Carry-Blair media (Oxoid, England) and kept chilled in an ice box 4℃ during transport to the laboratory at the Faculty of Veterinary Medicine, Khon Kaen University Thailand, for isolation and identification.

Salmonella spp. isolation and identification: Isolation and identification were performed according to the ISO 6579:2002/AMD 1:2007 procedure. Briefly, each swab sample was placed in 9 ml of buffer peptone water (BPW, Oxoid) for the pre-enrichment step. After incubation at 37°C for 18 to 24 h, 3 loopfuls of BPW culture were transferred to modified semisolid Rappaport-Vassiliadis medium (MSRV, Oxoid) and then incubated at 42°C for 18 to 24 h. Positive colonies were streaked on Xylose-Lysine-Desocholate agar (XLD; Oxoid) and Hektoen Enteric agar (HE; Oxoid) and incubated at 42 C. The plates were inspected after 24 h and positive colonies were placed on Triple Sugar Iron agar (TSI; Oxoid) and Motility Indole-Lysine agar (MIL; Oxoid). Colonies, either from XLD or HE, which tested positive on both TSI and MIL were identified as *Salmonella* spp.

Serotyping: *Salmonella* serotype identification was conducted at Department of Medical Science, Ministry of Public Health, Thailand. The slide agglutination method was carried out with the commercial antisera according to the manufacturer's instructions (S&A Reagents Ltd, Bangkok, Thailand) to detect the presence of O (somatic) and H (flagellar) antigens. The results were interpreted following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).

Antimicrobial susceptibility testing: The isolates were tested for antimicrobial sensitivity against ampicillin (AMP, 10 µg), amoxycillin/clavulanate (AMC, 20/10 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), cefotazidime (CAZ, 30 µg), nalidixic acid (NAL, 30 µg), norfloxacin (NOR, 10 µg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg), and tetracycline (TET, 30 µg) by disk diffusion test (Oxoid; Hampshire, England) according to the National Committee for Clinical and Laboratory Standard Institute (CLSI, 2018). *Escherichia coli* ATCC® 25922 was used as a quality control strain.

Statistical analysis: *Salmonella* prevalence, pattern of antimicrobial resistance rate and seasonal serovars distribution were analyzed and presented descriptively. The association of *Salmonella* positive proportion and antimicrobial resistance rate between species, markets and seasons was analyzed by Chi-square test using the SAS statistical package (version 9.1.3, SAS Institute, Inc., 2002–2003, Cary, NC, USA). A *P*-value ≤ 0.05 was considered statistically significant.

Results

The prevalence of *Salmonella* contamination isolated from pork, beef and chicken meat at retail markets in Vientiane capital, Lao PDR were 49.2%, 61.7% and 61.3% respectively (Table 1). Prevalence of *Salmonella* was not significantly difference between the 3 markets (data not showed). Percentage of *Salmonella* contamination in beef (95.2%) and chicken meat (77.3%) was significantly highest in rainy season (P = 0.0003, P =0.0432, respectively).

Thirty-nine serotypes of 242 *Salmonella* isolates from pork, beef and chicken meat were found. The most common serotype in pork, beef and chicken meat was *S.* Rissen (47/127, 37.0%), *S.* Derby (7/65, 10.8%) and *S.* Corvallis (19/50, 38.0%), respectively. High number of serotypes Rissen in pork were found in winter and summer season. Serotype Derby from beef isolates were high number in winter season. Isolates from chicken meat found serotype Covallis were high in winter and summer season (Table 2).

In general, for all *Salmonella* isolated (n = 242), resistance against TET (72.3%) was found at the highest frequency, followed by AMP (62%), SXT (51.7%) and CHL (26%). Similar resistant pattern was observed for TET, AMP and SXT, i.e. significantly higher in pork than beef and chicken meat, respectively (Figure 1).

Seasonal variation of resistant isolates was observed in all antimicrobial tested. However, resistant rate against TET was rather high in all seasons (74%, 73%, 69% in rainy, winter and summer, respectively). All isolates from pork, beef and chicken meat were susceptible to AMC, CIP and NOR in rainy season. In winter season, all isolates were susceptible to CIP and NOR. For all positive isolate, MDR (being resistance to at least three different antimicrobial classes) were found in beef (2.54%), chicken meat (29.23%) and pork (73.23%).

Table 1 Prevalence of *Salmonella* contamination isolated from pork, beef and chicken meat

Samples	Number	Positive (%)	Rainy (%)	Winter (%)	Summer (%)
pork	258	127 (49.2)	39/79 (49.4)	55/100 (55.0)	33/79 (41.8)
beef	81	50 (61.7)	20/21 (95.2)[a]	21/36 (58.3)[b]	9/24 (37.5)[b]
chicken meat	106	65 (61.3)	17/22 (77.3)[a]	32/49 (65.3)[b]	16/35 (45.7)[b]
Total	445	242 (54.4)	76/122 (62.3)	108/185 (58.4)	58/138 (42.0)

Different superscript letters between column in the same line are significantly different ($p \leq 0.05$)

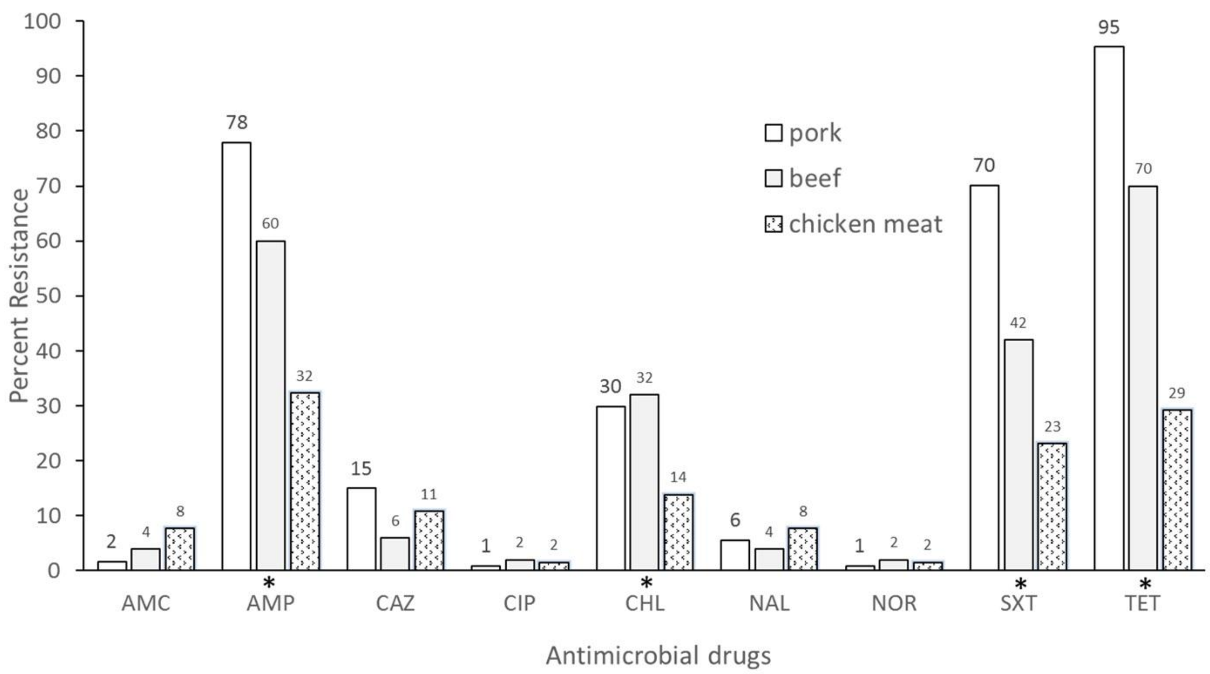

Figure 1 Antimicrobial resistance patterns of *Salmonella* in pork, beef and chicken meat
*indicated significant different between meat types ($p \leq 0.05$)

Table 2 Serotypes of *Salmonella* isolated from pork, beef and chicken meat

Sample	Serotypes of *Salmonella* (number)		
	rainy	winter	summer
pork	Meleagridis (19), Rissen (6), Anatum (5), Stanley (3), Typhimurium (2), Derby (2), Agona (1), Monschaui (1)	Rissen (21), Kedougou (10), Goldcoast (6), Derby (5), Stanley (3), London (3), Worthington (3), Anatum (2), Typhimurium (1), Bareilly (1)	Rissen (20), Stanley (3), Goldcoast (2), London (2), Corvallis (1), Gaminara (1) enterica ser. 1,4,5,12:d:- (1), enterica ser. 4,12:i:- (1), enterica ser. 4,5,12:d:- (1), enterica ser. 6,7,e,h:- (1),
beef	Meleagridis (5), Anatum (2), Weltevreden (2), Typhimurium (2), Derby (2), Stanley (1), Newport (1), Bovismorbificans (1), London (1), Lexington (1), Caen (1), Wandsworth (1)	Derby (7), Stanley (4), Kedougou (3), Rissen (2), London (2), Typhimurium (1), Altona (1), Anatum (1)	Rissen (4), Hvittingfoss (2), Kentucky (1), enterica ser. 8,20:-:- (1), London (1)
Chicken meat	Kentucky (7), Agona (4), Stanley (3), Altona (1), Mbandaka (1), enterica ser. 4,5,12:b:- (1),	Corvallis (13), Derby (3), Typhimurium (2), livingstone (2), Enteritidis (2), Stanley (1), Newport (1), Bareilly (1), Bovismorbificans (1), Rissen (1), Kentucky (1), Worthington (1), Kedougou (1), enterica ser. 4,5,12:b:- (1), salamae ser. 41:b:- (1)	Corvallis (6), Monschaui (4), Agona (1), Kentucky (1), Virchow (1), Concord (1), Muenster (1), Alachua (1)

Discussion

From this study, high *Salmonella* contamination in the 3 retail markets in Vientiane capital, Lao PDR was observed. According to the previous study, *Salmonella* spp. contamination in pork seem to be a consequence of food chain, i.e. infected pig to carcass contamination, as reported by Sinwat *et al* (2016). They reported that 38.7% of infected pig was found by rectal swab and increasing contamination was found by carcass swab (53.3%). Highest percentage of *Salmonella* spp. could be isolated from fresh market pork (72.3%). Sanitation practice of market could be the most important factor explanation to this high contamination. The lack of covering materials, unsuitable storage conditions and inadequate disinfection practices at the purchasing areas during the meat cutting and handline processes can substantially increase the risk of bacterial colonization.

Result from the present study showed that *Salmonella* contamination isolated from pork, beef and chicken meat at retail markets in Vientiane capital, Lao PDR were 49.2%, 61.7% and 61.3% respectively. Lower isolated found in pork compare to those found in beef and chicken meat probably in accordance with slaughter hygiene. For pork, the samples were collected from markets which received pork from the 2 big commercial slaughterhouses, but received from home slaughtering for beef. For chicken meat, some samples were native chicken meat which also be slaughtered by home slaughtering but some samples were imported from Thailand but finally probably be contaminated from the native chicken meat on stall in market.

In the present study, proportion of *Salmonella* contamination in beef (95.2%) and chicken meat (77.3%) were significantly highest in rainy season and lowest in summer (37.5% and 45.7%, respectively). The result was different from Zdragas *et al* (2012), in Greece, who reported that the highest isolation rate (60.4% was detected from chicken carcass during summer and the lowest (18.75%) isolation rate was detected during winter. This contamination trend was also different from those report for human salmonellosis (EFSA, 2009; 2011; 2012). Difference of seasonal factor such as local temperature or humidity was at least one explanation. Yun *et al*. (2016) found that the ambient temperature higher than 15 ℃ was positively correlated to incidences of *S.* Enteritidis and *S.* Typhimurium in the three reporting areas in Germany 2001-2004. In Lao PDA, average ambient temperature was rather high all year round (21.8-28.8℃) (Climate-data.org, 2021), especially quite high during rainy and summer season. Higher humidity during rainy season (83-86%) compare to summer season (58-77 %) in this area probably affect dominately on the *Salmonella* spp. contamination rate. In the present study, the seasonal variation could not be detected for *Salmonella* isolation from pork. The different pattern, i.e. seasonal variation of *Salmonella* spp. detection rate in beef and chicken meat but not in pork, in accordance with slaughter hygiene as mentioned before for the prevalence. Therefore, different slaughterhouse hygiene could be the explanation for the seasonal variation, i.e. high hygiene level for pork (commercial slaughterhouse, e.g. hanging slaughtering) and low hygiene level for beef and chick meat (home slaughtering, e.g. floor slaughtering).

Home or traditional slaughtering is generally performed within the household, or outside registered slaughter facilities (abattoirs). According to EU standards, domestic (traditional) slaughter of animals is not acceptable, except in cases where such meat is intended for personal consumption. Increasing of meat contamination especially during rainy season due to high contaminate water source, wet condition/poor waste water drainage, high humidity and more vector such as cockroach, lizard, or flies. Bhavnani *et al*. (2014) suggested that the impact of rainfall patterns on diarrhea is likely to be most extreme when sanitation is compromised. Control and prevention of *Salmonella* contamination should be good management from farm to table such as standard farm, hygiene slaughterhouse, sanitation and hygiene of market until cooked well (Angkititrakul *et al*., 2005).

The most common serotype isolated from pork (Rissen, 37.0%), beef (Derby, 10.8%) and chicken meat (Corvallis, 38.0%) were found. In pork, S. Typhimurium and S. Rissen are the majority serotypes identified in the other reports (Patchanee *et al*., 2016; Sinwat *et al*., 2016). However, in beef, S. Stanley and S. Typhimurium have been reported as the most common serotypes (Boonmar *et al*., 2013) which differed from the present study. Factors influencing serotype distribution have not been confirmed. Covalis was also the common serotype found in chicken meat by Meunsene *et al*. (2021) (44.19%) and Trongjit *et al*. (2017) (5.5%). Seasonal serotype variation was observed in all 3 meat types. High number of serotypes in beef, chicken meat and pork were found in rainy (11); winter (15); winter (10) and summer (10), respectively.

The result from the present study shown that AMP and TET were the highest resistant for *Salmonella* isolate from pork, beef and chicken meat. The result was concordance to the report previously in Laos PDR for the 3 meat types (17%, 33%) in pork, 8%, 19% in beef and 10%, 6% in chicken meat) (Meunsene *et al*., 2021). However, higher resistant rate of AMP and TET was observed in the present study. Isolates from pig carcass at slaughterhouse in Vientiane, Lao PDR also found high resistant to TET (97.6%) and AMP (91.5%) (Sinwat *et al*., 2016). According to Zdragas *et al*. (2012), high resistant to both TET (56.2%) and AMP (33.3%) from chicken carcasses also found in Greece and the uncontrolled use of antimicrobials in poultry production was suspected. Therefore, high resistance to both AMP and TET in Laos PDR in this study may indicated inappropriate antimicrobial used in farm animals especially AMP and TET.

Seasonal variation of resistant isolates was observed in all antimicrobial tested. However, seasonal factors influence antimicrobial use or resistance needed for further study. In the present study, higher prevalence of MDR in pork than beef and chicken meat isolates were also highlighted.

The results indicated cross-contamination among meat types and re-contamination by *Salmonella* spp. could occurred in meat at the retail markets in Laos. Hygiene and sanitation of slaughterhouse and retail market should be concerned to control and prevent of *Salmonella* spp. contamination along the food supply chain.

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