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Prevalence of *Listeria monocytogenes, E. coli, Salmonella* Spp. and *Staphylococcus aureus* Bacteria Contamination on Meat at Public Market in the North of Vietnam

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Abstract

480 (four hundred and eighty) samples of beef, pork, and poultry sold at public markets in Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen were collected in the two years of 2015 and 2016 to determine the contamination of *Listeria monocytogenes, Staphylococcus aureus, E. coli*, and *Salmonella* spp. the results showed that: The beef samples contaminated with *Listeria monocytogenes* was 10.0%; *Staphylococcus aureus* 21.6%, *E. coli* 65.0%; and *Salmonella* spp. 15.0%; The pork samples contaminated with *Listeria monocytogenes* was 11.6%, *Staphylococcus aureus* 20.0%, *E. coli* 63.3%; and *Salmonella* spp. 11.6%; The poultry samples contaminated with *Listeria monocytogenes* was 8.3%; *Staphylococcus aureus* 15.0%, *E. coli* 43.3%, and *Salmonella* spp. 10.0%.

Staphylococcus aureus bacteria bearing genome sequence of enterotoxin typical B accounted for 79.4%; verotoxin-producing of *Escherichia coli* genome sequence of VT1 (verotoxin 1) accounted for 11.8%, and VT2 (verotoxin 2) accounted for 9.4%; *Stn* (heat-stable enterotoxin gene) enterotoxin genome sequence of *Salmonella* spp. accounted for 78.2%, *InvA* (Invasion gene A) 60.8%; *hlyA* (listeriolysin O-encoding gene) genome sequence of *Listeria monocytogenes* contamination on beef accounted for 21.8% (beef), 22.5% (pork), 34.3% (poultry).

The *Listeria monocytogenes* were resistant to amoxicilline (77.7%), nitrofurantoin, ceftazidime, and oxytetracycline (3.7%), and 11.1% were resistant to Erythromycin; *Staphylococcus aureus* were resistant to amoxicilline (82.7%), nitrofurantoin (4.3%), erythromycin (15.5%), and 3.4% were resistant to ceftazidime; *E. coli* were resistant to ceftazidime (3.7%), kanamycin (7.5%), rifampicin (6.3%), bacitracin (48.1%), and 1.2% were resistant to oxytetracycline; *Salmonella* spp. bacteria isolates were resistant to kanamycin, ceftazidime (6.5%), and 63.0% were resistant to bacitracin.

Keywords: Bacteria; Meat; Virulence

Introduction

According to World Health Organization, every year, there are at least 600 million people all over the world (around 1/10 of the world's population) suffer from food poisoning, with 420,000 deaths, and most of the victims are children [40]. In Vietnam, every year, there were from 250 to 500 cases of food poisoning, affecting 7,000 to 10,000 people; of which from 100 to 200 victims are dead. Of the causes of food poisoning, the ones caused by micro organisms account for 33% to 49% [14]. Ono HK, et al. stated that globally, food poisoning caused by micro organisms account for 70% [31]. The main bacteria causing food poisoning include *Escherichia coli, Salmonella* spp. *Listeria monocytogenes, Clostridium perfringens*, and *Staphylococcus aureus* [2]. Ateba CN, et al. successfully isolated *Escherichia coli* O157:H7 bacteria carrying virulence gene from pork (67.7%), beef (27.7%), used water (2.3%) and from human (0.77%); Abouzeed YM, et al. discovered that the rate of *Salmonella typhimurium* on the beef samples studied was 4.6%; Swati Singh found 10.66% of the buffalo meat samples contaminated with *Salmonella* spp [41,5].

According to the results of the study by Mengesha D, et al. the levels of contamination of *Listeria monocytogenes* was 62.5% on pork, 47.7% on beef, 16.0% on chicken; and 42.7% on ice-cream and Robin LT, et al. stated that *Salmonella* spp. *Listeria monocytogenes*, and *Staphylococcus aureus* bacteria on meat were the leading cause for food poisoning [4,5,11-14,17,24,25,27,38]. Kinga Wieczorek, et al. said that L*isteria monocytogenes* isolated from beef belong to a group with the virulence which can cause diseases on human, and which was resistant to antibiotics i.e. oxacillin (72.2%) and clindamycin (37.0%) [21].

Kwon NH, et al. confirmed that *Staphylococcus aureus* isolated from slaughter houses produced entertoxin type B [22]. Reyad R Shawish, et al. declared that in Saudi Arabia and Egypt, *Staphylococcus aureus* were found on 12% to 38% of the food made from beef [37]. They also revealed that the sources of infection included contaminated cattles, waste, soil, air, tools, slaughtering workers, and unhygienic processing.

Materials and Methods

Materials

- Beef, pork, poultry (chicken and duck) sold in Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen markets.

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• *Listeria monocytogenes, E. coli, Salmonella* spp, and *Staphylococcus aureus* bacteria isolates (The *Listeria monocytogenes, E. coli, Salmonella* spp, and *Staphylococcus aureus* bacteria were isolated from the meat samples collected).

• Ordinary and specific environment for culturing, separating, selecting and examining L*isteria monocytogenes, E. coli, Salmonella* spp, and *Staphylococcus aureus.*

Study sites

• Sample collection: Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen markets.

• Sample examination: Institute of Life Science, Thai Nguyen Agriculture and Forestry University; Institute of Genome Research, Vietnam Academy of Science and Technology.

Methods

The samples were collected from the markets according to ISO 17604:2003 on Meat and meat products – Sample collection and preparation. Part 1: Sample collection.

Isolation and identification of *Salmonella* from meat, poultry and egg products. Australian Standard, Approved methods for testing of meat & meat products [15].

Enumeration of total aerobic bacteria and *Escherichia coli* in minced meat and on carcass surface samples with an automated most-probable-number method compared with colony count protocols [33].

Bacteriological Analytical Manual Chapter 12: *Staphylococcus aureus*, U.S. Department of Health and Human Services, and Microbiological Methods & Bacteriological Analytical Manual (BAM) [36].

Bacteriological Analytical Manual Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria, U.S. Department of Health and Human Services, Microbiological Methods & Bacteriological Analytical Manual (BAM), General guidance for enumeration of presumptive Escherichia coli - Most probable number technique [34]. Approved methods for testing of meat & meat products [6].

Bacterial serotyping methods the determination of the serotype of *Salmonella* and *E. coli* strains. The agglutination methods used were based on those described by Quinn PJ, et al. [35].

Determination of the prevalence of *Listeria monocytogenes* according to Isolation and identification of *Listeria monocytogenes* from red meat, poultry, egg, and environmental samples, Australian Standard [16].

Examination of the biological and chemical characteristics of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* isolates according to: Selecting 30 strains isolated from the contaminated samples of beef, pork, and poultry which did not meet hygien standard [35].

Multiplex polymerase chain reaction analysis of the targeted genes of interest was performed using Dream Taq DNA polymerase (Thermo Scientific, USA). For the amplification, five microlitres of DNA was added to 20 µL of master mix containing 12.5 µL of DreamTaq DNA polymerase (2X DreamTaq Green Buffer, dATP, dCTP, dGTP, and dTTP, 0.4 mM each, and 4 mM MgCl2) (Thermo Scientific, USA), 0.5 µL (0.2 µM) of respective oligonucleotide primers and the reaction volume was made up with nuclease free water. PCR was performed in a thermal cycler (Bio-Rad Laboratories, USA). The amplification cycles consisted of an initial DNA denaturation at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 45 s, primer annealing at 55°C, for 45 s, extension at 68°C for 2 min, and a final single elongation at 72°C for 5 min. The primers used to amplify the targeted genes were as previously reported by Institute of Genome Research, Vietnam Academy of Science and Technology and are summarized in Table 1, 2, 3 and 4. Negative controls, substituting DNA template with ultrapure water (Sigma-Aldrich, UK), were included in all PCR runs. Amplified DNA was resolved by 2% agarose gel electrophoresis and visualised under UV transillumination.

Table 1: Primers for determining the encoded gene producing VT1 AND VT2 of *Escherichia coli* (Source: Institute of Genome Research, Vietnam Academy of Science and Technology)

Primers	Primer sequences	Size of sequences (bp)
VT1-F	5'-CAC CAG ACA ATG TAA CCG CTG-3'	348
VT1-R	5'-CAG TTA ATG TGG TGG CGA AGG- 3'	
VT2-F	5'-GCG TCA TCG TAT ACA CAG GAG C-3'	584
VT2-R	5'-ATC CTA TTC CCG GGA GTT TAC G-3'	

Table 2: Primers for determining the encoded gene producing *InvA*, *Stn* of *Salmonella* spp. (Source: Institute of Genome Research, Vietnam Academy of Science and Technology)

Primers	Primer sequences	Size of sequences (bp)
InvA	F: GTG AAA TTA TCG CCA CGT TCG GGC AA	521
	R: TCA TCG CAC CGT CAA AGG AAC C	
Stn	F: CTT TGG TCG TAA AAT AAG GCG	259
	R: TGC CCA AAG CAG AGA GAT TC	

Table 3: Primers for determining the encoded gene producing SEB of *Staphylococcus aureus* (Source: Institute of Genome Research, Vietnam Academy of Science and Technology)

Primers	Primer sequences	Size of sequences (bp)
SEB - F	ccg GAATTC atg CCA GAT GAG TTG CAC AAA	534
SEB - R	ccc AAGCTT tca TCC CGT TTC ATA AGG CGA	

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Table 4: Primers for identifying encoded gene hly producing Listeriolysin of *Listeria monocytogenes* (Source: Institute of Genome Research, Vietnam Academy of Science and Technology)

Primers	Primer sequences	Size of sequences (bp)
hly – F	5`- GCAGTTGCAAGCGCTTGGAGTGAA- 3`	456
hly – R	5`- GCAACGTATCCTCCAGAGTGATCG- 3`	

Antibiotic susceptibility testing was performed by the Kirby-Bauer disc-diffusion test, which conforms to the recommended standard as described by Quinn PJ, et al. [35]. Briefly, an inoculum of each pure bacterial isolate was emulsified in 3 mL of sterile normal saline and the density adjusted to 0.5 McFarland standard. A sterile cotton swab was dipped into the standardized suspension of bacterial cultures and used to inoculate Mueller-Hinton Agar (MHA) plates (Oxoid, England), and the plates were allowed to dry. Antibiotic discs with the following drug contents amoxicillin, nitrofurantoin, ciprofloxacin, bacitracin, erythromycin, oxytetracycline, ceftazidime, nalidixic acid, gentamicin, vancomycin, oxacillin, kanamycin, and rifampicin, (Antibiotic Becton, Dickson and Company, Sparks, USA; Le Pont de Claix, France) were placed onto MHA plates. The plates were incubated at 37°C for 24 hours. The zone diameter was measured and results were interpreted [35]. The reference strains *E. coli, Salmonella, Listeria monocytogenes* and *Staphylococcus aureus* were used to verify the quality and accuracy of the testing procedure.

Biological statistic was processed with SPSS: Statistical analysis was performed using SPSS version 22.0 [39]. The chi-square test was used to compare rate of isolation of the various pathogens in beef, pork, poultry (chicken and duck) and the different study sites. Comparisons were also done among the markets. Differences were considered significant at $P < 0.05$.

Results

Sale of meat in retail markets in Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen

A survey was conducted on the sale of cattle and poultry fresh meat at public markets (public market) in Northern mountaineous provinces of Vietnam, including Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen with sample collection time in a day, number of shops, rate of controlled slaughtering meat, number of cattle and poultry killed, and quantity of meat sold per day. The results are presented in table 5.

From table 5, it is clear that all the markets operated in the same time frame, from 6:00 am to 19:00 pm. However, the number of shops varied. In Bac Giang, there were 18 beef shops with the sale of around 2.39 tons per day, 49 pork shops with 4.16 tons per day, and 16 poultry meat shops selling roughly 0.56 ton per day. However, only 8% to 14% of the meat was under control (the cattle and poultry sources could be traced, and they were killed in slaughtering houses which met veterinary hygiene requirement); In Tuyen Quang there were 19 beef shops selling1.81 tons per day, 47 pork shops selling 3.23 tons per day, and 11 poultry meat shops selling 0.41 ton per day. However, only 8% to 12% of the meat was under control; In Lang Son there were 18 beef shops with the sale of 2.75 tons per day, 36 pork shops with 2.17 tons of meat being sold per day, and 12 poultry meat shops with 0.45 ton sold per day). Of which, only 6% to 11% was under control;

Table 5: Sales of cattle and poultry meat in Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen

Sample collection sites (market)	Sample collection time (hour of day)	Number of shops	Rate of controled slaughtering (%)	Number of cattle slaughtered	Quantity of meat sold
		$\overline{X} \pm m_{\overline{X}}$			
Beef					
Bac Giang	19-Jun	18 ± 1.1	14 ± 1.8	14.56 ± 2.1	2.39 ± 0.5
Tuyen Quang	19-Jun	19 ± 1.5	12 ± 2.2	10.05 ± 1.8	1.81 ± 0.1
Lang Son	19-Jun	18 ± 1.2	11 ± 2.1	12.36 ± 1.2	2.75 ± 0.1
Thai Nguyen	19-Jun	26 ± 2.1	15 ± 1.6	22.42 ± 1.5	4.96 ± 0.1
Pork					
Bac Giang	19-Jun	49 ± 1.1	12 ± 1.5	24.31 ± 1.3	4.16 ± 0.3
Tuyen Quang	19-Jun	47 ± 1.5	11 ± 2.1	18.15 ± 1.1	3.23 ± 0.6
Lang Son	19-Jun	36 ± 1.2	10 ± 1.6	16.22 ± 2.2	2.17 ± 0.2
Thai Nguyen	19-Jun	58 ± 2.1	11 ± 1.3	26.13 ± 1.2	3.69 ± 0.5
Poultry					
Bac Giang	19-Jun	16 ± 1.1	8 ± 1.3	280.36 ± 2.2	0.56 ± 0.6
Tuyen Quang	19-Jun	11 ± 1.5	8 ± 1.2	209.25 ± 1.5	0.41 ± 0.5
Lang Son	19-Jun	12 ± 1.2	6 ± 1.1	228.16 ± 1.3	0.45 ± 0.1
Thai Nguyen	19-Jun	18 ± 2.1	8 ± 1.2	321.32 ± 1.2	0.61 ± 0.4

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In Thai Nguyen there were 26 beef shops selling 4.96 tons per day, 58 pork shops selling 3.69 tons per day, and 18 poultry meat shops selling 0.61 ton per day. Yet only 8% to 15% of the meat was under control.

The results reflect the current situation of cattle and poultry slaughtering and meat selling in Vietnam, which were in line with the report of the Department of Animal Health, which states that by the end of 2015, in Vietnam, there were around 30,750 slaughter houses, 910 of which were concentrated and 100% were under the control of local department of animal health [9]. There were more than 29,840 small slaughter houses with the capacity of 1- 3 cattle or poultry per day, more than 8,000 of which were under control, accounting for 27%. Thus, nearly 22,000 small and scattered slaughter points were not under the control of local sub department of animal health.

Enumeration of Total Aerobic Bacteria

480 meat samples were collected in Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen markets for enumerating of total aerobic bacteria found in meat. The results are presented in table 6.

From table 6 we can see that all the meat samples were found contaminated with aerobic bacteria, varying in levels of infection. The samples contaminated with aerobic bacteria and did not meet hygiene standard on total aerobic bacteria accounted for 26.66% of Lang Son samples and 46.66% of Bac Giang ones (beef); 30.0% of the samples from Tuyen Quang and 48.33% of the ones from Bac Giang (pork); and 23.33% of the samples from Tuyen Quang and 36.66% of the ones from Bac Giang (poultry). The result of hypothesis check was $P > 0.05$. Thus, the difference of the levels of infection among the locations was not significant (relative statistical significance).

On the levels of contamination, on beef, the lowest total of aerobic bacteria found on the samples which did not meet hygiene standard was 1.2×10^5 CFU/gram (samples from Lang Son), and the lowest was 3.2×10^7 CFU/gram (samples from Bac Giang); on pork, the lowest level was 1.2×10^5 CFU/gram (samples from Thai Nguyen and Lang Son), and the lowest level were 2.6×10^7 CFU/gram (samples from Bac Giang); and on poultry, the lowest level was 1.1×10^5 CFU/gram (samples from Tuyen Quang), and the highest level was 1.6×10^6 CFU/gram (samples from Bac Giang).

The result of the survey on Enumeration of total aerobic bacteria on the meat samples showed us the hygienic quality of the meat sold in the markets. The results were in line with those of Enumeration on total aerobic bacteria on meat; Chicken Carcasses on the number of bacteria found on chicken meat; and on micro bacteria on beef in Kigali city, Rwanda [33, 17, 12].

Table 6: Total number of aerobic bacteria found in meat

Types of meat	Location of market	Factors					Levels of contamination of the samples not complying to hygienic standard		
		No. of samples tested	No. of positive samples	Rate (%)	No. of samples not complying to hygienic standard	Rate (%)	Lowest (CFU/g)	Highest (CFU/g)	Medium (CFU/g)
Beef	Bac Giang	120	120	100	56	46.66	1.3×10^6	3.2×10^7	1.2×10^7
	Tuyen Quang	120	120	100	38	31.66	1.8×10^5	2.4×10^7	2.3×10^7
	Lang Son	120	120	100	32	26.66	1.2×10^5	2.6×10^7	1.1×10^7
	Thai Nguyen	120	120	100	46	38.33	1.7×10^5	2.2×10^7	1.3×10^7
Pork	Bac Giang	120	120	100	58	48.33	1.8×10^6	2.6×10^7	1.2×10^7
	Tuyen Quang	120	120	100	36	30.0	1.3×10^5	2.1×10^7	2.3×10^7
	Lang Son	120	120	100	44	36.66	1.2×10^5	2.2×10^7	2.1×10^7
	Thai Nguyen	120	120	100	46	38.33	1.2×10^5	1.3×10^7	1.1×10^7
Poultry	Bac Giang	120	120	100	44	36.66	1.5×10^6	1.6×10^7	1.2×10^7
	Tuyen Quang	120	120	100	28	23.33	1.1×10^5	2.1×10^7	1.3×10^7
	Lang Son	120	120	100	30	25.0	1.5×10^5	1.3×10^7	1.2×10^7
	Thai Nguyen	120	120	100	36	30.0	1.3×10^5	2.2×10^7	1.2×10^7

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Prevalence of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* from meat

Detection of *Listeria monocytogenes*, *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* in beef

Table 7 shows the result of prevalence of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in the beef sold at public markets in Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen. The details are as follows:

From table 7, it is clear that the beef samples contaminated with *Listeria monocytogenes* accounted for 5.0% of the samples from Tuyen Quang and Lang Son, 6.66% of the samples from Thai Nguyen, and 10.0% of the samples from Bac Giang. According to hygenic standard the samples contaminated with *Listeria monocytogenes* did not meet hygiene standards (*Listeria monocytogenes* should not be found on 25gram product). The figures were lower that those of Occurrence and distribution, with *Listeria monocytogenes* on beef accounted for 47.7% [24].

The contamination rate of *Staphylococcus aureus* on beef was 16.66% of the samples from Tuyen Quang and 21.66% of the samples from Lang Son and Thai Nguyen, of which from 10.0% to 15.0% of the samples did not meet hygiene standard (*Staphylococcus aureus* on meat ≤10^2CFU/gram product). The results were in line with those in Saudi Arabia and Egypt with 12% to 38% of the beef samples found contaminated with *Staphylococcus aureus* [37].

Prevalence of *E. coli* showed that infection on beef was 53.33% of the samples from Tuyen Quang and 65.0% of the samples from Bac Giang, of which 25.0% of the samples from Lang Son and 30.0% of the samples from Bac Giang did not meet hygiene standard (*E. coli* on meat ≤10^2CFU/gram product). The results were in line with those of, with Escherichia coli isolated on pork (67.7%), beef (27.7%), used water (2.3%) and from human (0.77%) [5].

Salmonella spp. on beef accounted for 10.0% of the samples from Tuyen Quang and 15.0% of the samples from Bac Giang. According to hygenic standard, the samples contaminated with *Salmonella* spp. did not meet hygiene standard (*Salmonella* spp. should not be found on 25gram product). The results were in line with those Characterization of *Salmonella typhimurium* found on beef was 4.6% [1].

Table 7: Prevalence of *Listeria monocytogenes, E. coli, Salmonella spp.* and *Staphylococcus aureus* in beef

Type of bacteria	Market site	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples not complying to hygenic standard	Rate (%)
Listeria monocytogenes	Bac Giang	120	12	10.0	12	10.0
	Tuyen Quang	120	6	5.0	6	5.0
	Lang Son	120	6	5.0	6	5.0
	Thai Nguyen	120	8	6.66	8	6.66
Staphylococcus aureus	Bac Giang	120	24	20.0	16	13.33
	Tuyen Quang	120	20	16.66	12	10.0
	Lang Son	120	26	21.66	16	13.33
	Thai Nguyen	120	26	21.66	18	15.0
E. coli	Bac Giang	120	78	65.0	36	30.0
	Tuyen Quang	120	64	53.33	32	26.66
	Lang Son	120	76	63.33	30	25.0
	Thai Nguyen	120	70	58.33	32	26.66
Salmonella spp.	Bac Giang	120	18	15.0	18	15.0
	Tuyen Quang	120	12	10.0	12	10.0
	Lang Son	120	16	13.33	16	13.33
	Thai Nguyen	120	14	11.66	14	11.66

Detection of Listeria *monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in pork

Table 8 shows the results of prevalence of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in pork sold at public markets in Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen. The details are as follows:

From table 8, it is clear that pork contaminated with *Listeria monocytogenes* accounted for 5.0% of the samples from Tuyen Quang and 11.66% of the samples from Lang Son. According to hygenic standard, the samples contaminated with *Listeria monocytogenes* did not meet hygiene standard (*Listeria monocytogenes* should not be found on 25gram product). The results were lower than those of Occurrence and distribution with *Listeria monocytogenes* contamination on 62.5 of the pork samples [24].

Staphylococcus aureus found on pork was 15.0% on samples from Lang Son to 20.0% of the samples from Bac Giang), of which

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6.66% of the samples from Tuyen Quang to 13.33% of the samples from Bac Giang) of the samples did not meet hygiene standard (*Staphylococcus aureus* on meat ≤10^2CFU/gram product). The results were in line with those on the number of S*taphylococcus aureus* on food sold at markets in Italia; on the contamination of *Staphylococcus aureus* on meat and food products [29,30,27].

E. coli found on beef was 50.0% of the samples from Tuyen Quang to 63.33% of the samples from Lang Son and Thai Nguyen), of which from 30.0% of the samples from Tuyen Quang to 43.33% of the samples from Bac Giang) did not meet hygiene standard (*E. coli* on meat ≤ 10^2CFU/gram product). The results were in line with those of, with Escherichia coli isolated on pork (67.7%), beef (27.7%), used water (2.3%) and on human (0.77%) [5].

Salmonella spp. found on pork was 5.0% of the samples from Tuyen Quang to 11.66% of the samples from Bac Giang), and according to hygenic standard the samples contaminated with *Salmonella* spp. did not meet hygiene standard (*Salmonella* spp. should not be found on 25gram product). The results were similar to the findings in report on the contamination of *Salmonella* spp. on the pork samples in Hanoi and Ho Chi Minh City published by World Bank [9].

Table 8: Prevalence of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in pork

Type of bacteria	Market site	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples not complying to hygenic standard	Rate (%)
Listeria monocytogenes	Bac Giang	120	8	6.66	8	6.66
	Tuyen Quang	120	6	5.0	6	5.0
	Lang Son	120	14	11.66	14	11.66
	Thai Nguyen	120	12	10	12	10.0
Staphylococcus aureus	Bac Giang	120	24	20.0	16	13.33
	Tuyen Quang	120	20	16.66	8	6.66
	Lang Son	120	18	15.0	10	8.33
	Thai Nguyen	120	20	16.66	12	10.0
E. coli	Bac Giang	120	64	53.33	52	43.33
	Tuyen Quang	120	60	50.0	36	30.0
	Lang Son	120	76	63.33	42	35.0
	Thai Nguyen	120	76	63.33	46	38.33
Salmonella spp.	Bac Giang	120	14	11.66	14	11.66
	Tuyen Quang	120	6	5.0	6	5.0
	Lang Son	120	8	6.66	8	6.66
	Thai Nguyen	120	12	10.0	12	10.0

Detection of *Listeria* monocytogenes, *E. coli, Salmonella* spp. and *Staphylococcus aureus* on poultry

Table 9 shows the result of prevalence of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in poultry sold at public markets in Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen. The details are as follows:

From table 9, it is clear that poultry contaminated with *Listeria monocytogenes* accounted for 5.0% on samples from Lang Son to 8.33% on samples from Thai Nguyen, and according to hygenic standard the samples contaminated with *Listeria monocytogenes* did not meet hygiene standard (*Listeria monocytogenes* should not be found on 25gram product). The results were lower than those of Occurrence and distribution, with *Listeria monocytogenes* on chicken was 16.0% [24].

Staphylococcus aureus found on poultry was 11.6% on samples from Lang Son and 15.0% of the samples from Bac Giang. Of which, 5.0% of the samples from Tuyen Quang and 11.66% of the samples from Bac Giang hygiene standard (S*taphylococcus aureus* on meat ≤ 10^2CFU/gram product). The results were in line with those on the number of *Staphylococcus aureus* on food sold at markets in Italia and on the contamination of *Staphylococcus aureus* on meat and food products [29,30].

Prevalence of *E. coli* showed that the infection on poultry was 30.0% of the samples from Thai Nguyen and Lang Son and 43.33% of the samples from Bac Giang. Of which, 11.66% on samples from Thai Nguyen and 15.0% of the samples from Tuyen Quang and Bac Giang did not meet hygiene standard (*E. coli* on meat ≤ 10^2CFU/gram product). The results were similar to the finding Enumeration of total *Escherichia coli* on pork, beef, and on other home animals [5].

Salmonella spp. found on pork accounted for 6.66% of the samples from Thai Nguyen, 10.0% of the samples from Lang Son and Tuyen Quang and 16.66% of the samples from Bac Giang. According to hygenic standard, the samples contaminated with *Salmonella* spp. did not meet hygiene standard (*Salmonella* spp. should not be found on 25gram product). The results were similar to the finding the contamination of *Salmonella* spp. on chicken meat samples [42,28].

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Table 9: Prevalence of *Listeria monocytogenes, E. coli, Salmonella spp. and Staphylococcus aureus* in poultry

Type of bacteria	Market site	No. of samples	Positive	Rate (%)	No. of samples not complying to hygenic standard	Rate (%)
Listeria monocytogenes	Bac Giang	120	8	6.66	8	6.66
	Tuyen Quang	120	8	6.66	8	6.66
	Lang Son	120	6	5	6	5.0
	Thai Nguyen	120	10	8.33	10	8.33
Staphylococcus aureus	Bac Giang	120	18	15	14	11.66
	Tuyen Quang	120	16	13.33	6	5.0
	Lang Son	120	14	11.66	10	8.33
	Thai Nguyen	120	16	13.33	8	6.66
E. coli	Bac Giang	120	52	43.33	18	15.0
	Tuyen Quang	120	42	35	18	15.0
	Lang Son	120	36	30	16	13.33
	Thai Nguyen	120	36	30	14	11.66
Salmonella spp.	Bac Giang	120	20	16.66	20	16.66
	Tuyen Quang	120	12	10	12	10.0
	Lang Son	120	12	10	12	10.0
	Thai Nguyen	120	8	6.66	8	6.66

Prevalence of *Listeria* monocytogenes, *E. coli, Salmonella* spp. and *Staphylococcus aureus* on meat by different sample collection time during the day

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in beef by different sample collection time

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in beef sold at the markets by different sample collection time during the day (from 6h to 11h, from 14h to 16h, and from 17h to 19h). The results were presented in table 10.

From table 10, we can see that the prevalence of *Listeria monocytogenes, E. coli, Salmonella* spp. and S*taphylococcus aureus* found in beef varied according to the sample collection time during the day. The details are as follows:

Listeria monocytogenes on beef in the time frame of 6h-11h was 10.0% of the samples from Thai Nguyen and Tuyen Quang, 15.0% of the samples from Lang Son and Bac Giang; in the time frame of 14h-6h, the total bacteria count was 5.0% of the samples from Thai Nguyen and Tuyen Quang and 10.0% of the samples from Bac Giang; from 17h to 19h *Listeria monocytogenes* on beef was 5.0% of the samples from Thai Nguyen and Bac Giang. With $P < 0.05$, we can see a statistical variance on that the differences were statistically significant on contamination of *Listeria monocytogenes* on beef in different time frame of sample collection during the day. The results were lower on that *Listeria monocytogenes* contamination, with 40% of the samples found positive on poultry, red meat and meat products; and with 47.7% of the beef samples found positive with *Listeria monocytogenes* [20,24].

Staphylococcus aureus: The contamination on beef in the time frame of 6h-11h ranged from 30.0% of the samples from Bac Giang to 45.0% of the samples from Lang Son; in the time frame of 14h to 16h, The contaminated samples ranged from10.0% of the samples from Tuyen Quang to 15.0% of the samples from Bac Giang, Thai Nguyen and Lang Son; in the time frame of from 17h to 19h , contamination rates ranged from 5.0% of the samples from Tuyen Quang and Lang Son to 10.0% of the samples from Thai Nguyen, and 15.0% of the samples from Bac Giang. Similar to *Listeria monocytogenes* with $P < 0.05$ we can see that the differences were statistically significant on contamination of *Staphylococcus aureus* on beef in different time frames of sample collection during the day. The results were in Saudi Arabia and Egypt with 12% to 38% of the beef samples found possitive with *Staphylococcus aureus* [37].

E. coli: The contamination in the time frame of 6h-11h ranged from 60.0% of the samples from Tuyen Quang to 75.0% of the samples from Thai Nguyen, Lang Son and Bac Giang; in the time frame of 14h to 16h, the contaminated samples ranged from 55.0% of the samples from Tuyen Quang to 65.0% of the samples from Thai Nguyen, Lang Son and Bac Giang; in the time frame of from 17h to 19h , contamination of *E. coli* on beef ranged from 35.0% of the samples from Thai Nguyen to 55.0% of the samples from Bac Giang. With $P < 0.05$ we can see that the differences were statistically significant on contamination of *E. coli* on beef in different time frame of sample collection during the day. The results were in successfully isolated *Escherichia coli* from beef (27.7%) and used water (2.3%) [5].

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Table 10: Prevalence of *Listeria monocytogenes, E. coli, Salmonella spp.* and *Staphylococcus aureus* in beef by sample collection time

Type of bacteria	Market site	Sample collection time								
		From 6h to 11h			From 14h to 16h			From 17h to 19h		
		No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)
Listeria monocytogenes	Bac Giang	40	6	15	40	4	10.0	40	2	5.0
	Tuyen Quang	40	4	10	40	2	5.0	40	0	0
	Lang Son	40	6	15	40	0	0	40	0	0
	Thai Nguyen	40	4	10	40	2	5.0	40	2	5.0
Staphylococcus aureus	Bac Giang	40	12	30	40	6	15.0	40	6	15.0
	Tuyen Quang	40	14	35	40	4	10.0	40	2	5.0
	Lang Son	40	18	45	40	6	15.0	40	2	5.0
	Thai Nguyen	40	16	40	40	6	15.0	40	4	10.0
E. coli	Bac Giang	40	30	75	40	26	65.0	40	22	55.0
	Tuyen Quang	40	24	60	40	22	55.0	40	18	45.0
	Lang Son	40	30	75	40	26	65.0	40	20	50.0
	Thai Nguyen	40	30	75	40	26	65.0	40	14	35
Salmonella spp.	Bac Giang	40	10	25	40	6	15.0	40	2	5
	Tuyen Quang	40	10	25	40	2	5.0	40	0	0
	Lang Son	40	12	30	40	2	5.0	40	2	5
	Thai Nguyen	40	12	30	40	2	5.0	40	0	0

Salmonella spp.: The contamination rate of *Salmonella* spp. on beef in the time frame of 6h-11h ranged from 25.0% of the samples from Tuyen Quang and Bac Giang to 30.0% of the samples from Thai Nguyen, Lang Son; in the time frame of 14h to 16h, The contaminated samples were from 5.0% of the samples from Tuyen Quang, Lang Son, Thai Nguyen to 15.0% of the samples from Bac Giang; in the time frame of from 17h to 19h , contamination of *Salmonella* spp. on beef accounted for 5.0% of the samples from Bac Giang and Lang Son. With $P < 0.05$ we can see that the differences were statistically significant on contamination of *Salmonella* spp. on beef in different time frames of sample collection during the day. The results with 4.6% of the beef samples found possitive with *Salmonella typhimurium* [1].

Detection of *Listeria monocytogenes*, *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* in pork by sample collection time

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in pork sold at markets by different sample collection time during the day (from 6h to 11h, from 14h to 16h, and from 17h to 19h), the results were presented in table 11.

From table 11 we can see that the contamination of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* on pork varied by sample collection time during the day in studied sites (with $P < 0.05$ we can see that the differences were statistically significant). The details are as follows:

Listeria monocytogenes: The contamination rate of *Listeria monocytogenes* on pork in the time frame of 6h-11h ranged from 15.0% Bac Giang and Tuyen Quang to 20.0% of the samples from Lang Son and Thai Nguyen; in the time frame of 14h to 16h, The contaminated samples ranged from 5.0% of the samples from Bac Giang to 10.0% of the samples from Lang Son and Thai Nguyen. In Tuyen Quang, no contaminated sample was found; in the time frame of from 17h to 19h, the rate of contamination of *Listeria monocytogenes* on pork was 5.0% of the samples from Lang Son. In the other sites no contaminated sample was found. The results were in line with those on contamination of *Listeria monocytogenes* on meat samples collected in 24 cities in China, with 5.4% to 37.8% of the samples contaminated [43].

Staphylococcus aureus: The contamination rate of *Staphylococcus aureus* on pork in the time frame of 6h-11h ranged from 25.0% of the samples from Lang Son to 40.0% of the samples from Bac Giang; in the time frame of 14h to 16h, the contaminated samples ranged from 10.0% of the samples from Thai Nguyen and Tuyen Quang to 15.0% of the samples from Bac Giang and Lang Son; in the time frame of from 17h to 19h , contamination of *Staphylococcus aureus* on pork ranged from 5.0% of the samples from Bac Giang, Lang Son and Thai Nguyen to 10.0% of the samples from Tuyen Quang, and 15.0% of the samples from Bac Giang. The results were in line with those on contamination of *Staphylococcus aureus* on food sold at markets in Italia [29,11].

E. coli: The contamination rate of *E. coli* on beef in the time

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Table 11: Prevalence of *Listeria monocytogenes, E. coli, Salmonella spp.* and *Staphylococcus aureus* in pork by sample collection time

Type of bacteria	Market site	Sample collection time								
		From 6h to 11h			From 14h to 16h			From 17h to 19h		
		No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)
Listeria monocytogenes	Bac Giang	40	6	15.0	40	2	5.0	40	0	0
	Tuyen Quang	40	6	15.0	40	0	0	40	0	0
	Lang Son	40	8	20.0	40	4	10.0	40	2	5.0
	Thai Nguyen	40	8	20.0	40	4	10.0	40	0	0
Staphylococcus aureus	Bac Giang	40	16	40.0	40	6	15.0	40	2	5.0
	Tuyen Quang	40	12	30.0	40	4	10.0	40	4	10.0
	Lang Son	40	10	25.0	40	6	15.0	40	2	5.0
	Thai Nguyen	40	14	35.0	40	4	10.0	40	2	5.0
E. coli	Bac Giang	40	32	80.0	40	20	50.0	40	12	30.0
	Tuyen Quang	40	28	70.0	40	18	45.0	40	14	35.0
	Lang Son	40	32	80.0	40	24	60.0	40	20	50.0
	Thai Nguyen	40	30	75.0	40	26	65.0	40	20	50.0
Salmonella spp.	Bac Giang	40	8	20.0	40	4	10.0	40	2	5.0
	Tuyen Quang	40	4	10.0	40	2	5.0	40	0	0
	Lang Son	40	6	15.0	40	2	5.0	40	0	0
	Thai Nguyen	40	6	15.0	40	4	10.0	40	2	5

frame of 6h-11h ranged from 60.0% of the samples from Tuyen Quang to 75.0% of the samples from Thai Nguyen, Lang Son and Bac Giang; in the time frame of 14h to 16h, The contaminated samples ranged from 55.0% of the samples from Tuyen Quang to 65.0% of the samples from Thai Nguyen, Lang Son and Bac Giang; in the time frame of from 17h to 19h , contamination of *E. coli* on beef ranged from 35.0% of the samples from Thai Nguyen to 55.0% of the samples from Bac Giang. The results were in line with *Escherichia coli* isolated from beef (27.7%), and used water (2.3%) [5].

Salmonella spp.: The contamination rate of *Salmonella* spp. on beef in the time frame of 6h-11h ranged from 25.0% of the samples from Tuyen Quang and Bac Giang to 30.0% of the samples from Thai Nguyen, Lang Son; in the time frame of 14h to 16h, The contaminated samples ranged from 5.0% of the samples from Tuyen Quang, Lang Son, Thai Nguyen to 15.0% of the samples from Bac Giang; in the time frame of from 17h to 19h , contamination of *Salmonella* spp. on beef accounted for 5.0% of the samples from Bac Giang and Lang Son. The results were in line with *Salmonella typhimurium* on beef was 4.6% [1].

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in poultry by sample collection time

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in poultry sold at markets was done on the samples collected at different time frames during the day (from 6h to 11h, from 14h to 16h, and from 17h to 19h), the results were presented in table 12.

From table 12 we can see that the contamination of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* on poultry varied by sample collection time during the day in studied sites ($P < 0.05$ we can see that the differences were statistically significant). The details are as follows:

Listeria monocytogenes: The contamination rate of *Listeria monocytogenes* on poultry in the time frame of 6h-11h ranged from 10.0% (Bac Giang and Lang Son) to 15.0% of the samples from Tuyen Quang and Thai Nguyen; in the time frame of 14h to 16h, contamination of the samples collected in all the site were similar, with 5.0%; in the time frame of from 17h to 19h, contamination of *Listeria monocytogenes* on pork was 5.0% of the samples from Thai Nguyen and Bac Giang. In the other sites, no contaminated sample was found. The results were in line with contamination of *Listeria monocytogenes* on meat samples collected in 24 cities in China with 5.4% to 37.8% [43].

Staphylococcus aureus: The contamination rate of *Staphylococcus aureus* on poultry in the time frame of 6h-11h ranged from 20.0% of the samples from Lang Son to 30.0% of the samples from Tuyen Quang and Thai Nguyen, in Bac Giang it was

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Table 12: Prevalence of *Listeria monocytogenes, E. coli, Salmonella sp. and Staphylococcus aureus* in poultry by sample collection time

Type of bacteria	Market site	Sample collection time								
		From 6h to 11h			From 14h to 16h			From 17h to 19h		
		No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)
Listeria monocytogenes	Bac Giang	40	4	10.0	40	2	5.0	40	2	5.0
	Tuyen Quang	40	6	15.0	40	2	5.0	40	0	0
	Lang Son	40	4	10.0	40	2	5.0	40	0	0
	Thai Nguyen	40	6	15.0	40	2	5.0	40	2	5.0
Staphylococcus aureus	Bac Giang	40	10	25.0	40	6	15.0	40	2	5.0
	Tuyen Quang	40	12	30.0	40	2	5.0	40	2	5.0
	Lang Son	40	8	20.0	40	4	10.0	40	2	5.0
	Thai Nguyen	40	12	30.0	40	4	10.0	40	0	0
E. coli	Bac Giang	40	24	60.0	40	16	40.0	40	6	30.0
	Tuyen Quang	40	26	65.0	40	10	25.0	40	6	15.0
	Lang Son	40	16	40.0	40	14	35.0	40	6	15.0
	Thai Nguyen	40	18	45.0	40	10	25.0	40	8	20.0
***Salmonella* spp.**	Bac Giang	40	8	20.0	40	9	22.5	40	3	7.5
	Tuyen Quang	40	6	15.0	40	5	12.5	40	1	2.5
	Lang Son	40	6	15.0	40	4	10.0	40	2	5.0
	Thai Nguyen	40	4	10.0	40	2	5.0	40	2	5.0

25.0%; in the time frame of 14h to 16h, the contaminated samples ranged from 5.0% of the samples from Tuyen Quang to 15.0% of the samples from Bac Giang, for samples from Thai Nguyen and Lang Son, 10.0% was contaminated; in the time frame of from 17h to 19h, contamination of *Staphylococcus aureus* on pork was 5.0% of the samples from Bac Giang, Lang Son and Tuyen Quang, no samples from Thai Nguyen was found with contamination. The results were in line with the contamination of *Staphylococcus aureus* on meat and food products [11,30].

E. coli: The contamination rate of *E. coli* on poultry in the time frame of 6h-11h ranged from 40.0% of the samples from Lang Son to 65.0% of the samples from Tuyen Quang; in the time frame of 14h to 16h, The contaminated samples were 25.0% of the samples from Tuyen Quang, Thai Nguyen to 40.0% of the samples from Bac Giang; in the time frame of from 17h to 19h , contamination of *E. coli* on poultry ranged from 15.0% of the samples from Lang Son and Tuyen Quang to 30.0% of the samples from Bac Giang. The results were in line on Enumeration of total *Escherichia coli* on meat and water (used for slaughtering and processing) [5].

Salmonella spp.: The contamination rate of *Salmonella* spp. on poultry in the time frame of 6h-11h ranged from 10.0% on samples from Thai Nguyen to 15.0% of the samples from Tuyen Quang, Lang Son and 20.0% of the samples from Bac Giang; in the time frame of 14h to 16h, The contaminated samples ranged from 5.0% on samples from Thai Nguyen to 10.0% of the samples from Lang Son,12.5% of the samples from Tuyen Quang, and 22.5% of the samples from Bac Giang: In the time frame of from 17h to 19h, contamination of *Salmonella* spp. on poultry ranged from 2.5% of the samples from Tuyen Quang, to 5% of the samples from Thai Nguyen and Lang Son, and 7.5% of the samples from Bac Giang. The results were similar to the findings of Characterization of *Salmonella* [1].

Prevalence of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in meat by seasons

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in beef by seasons

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in beef sold at markets by seasons (spring, summer, autumn, winter) in Bac Giang (BG), Tuyen Quang (TQ), Lang Son (LS), Thai Nguyen (TN), the results were

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presented in table 13.

From table 13 we can see the levels of contamination of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* on beef sold at the markets varied by seasons with higher contamination rates in spring and summer, and lower contamination rates in autumn and winter in the studied sites (with $P < 0.05$ we can see that the differences were statistically significant). The details are as follows:

Listeria monocytogenes on beef in Spring ranged from 10.0% (Tuyen Quang and Lang Son) to 43.3% (Thai Nguyen); 20% of the samples from Bac Giang were found contaminated. In summer, contamination of *Listeria monocytogenes* slightly reduced compared to the rate in spring ($P > 0.05$), with from 3.3% (Tuyen Quang) to 23.3% (Thai Nguyen). In autumn, contamination of *Listeria monocytogenes* on beef sharply decreased, especially in winter in comparison with the rate in spring ($P < 0.05$) with from 3.3% (Bac Giang, Tuyen Quang) to 6.6% (Thai Nguyen) of the samples found positive.

Staphylococcus aureus found on the beef samples in spring was 36.6% (Tuyen Quang) to 56.6% (Thai Nguyen); 43.3%, of the samples from Lang Son and 50.0% of the samples from Bac Giang was found possitive. In summer, contamination of S*taphylococcus aureus* on beef was slightly lower than the rates in spring ($P > 0.05$) with from 16.6% (Bac Giang) to 26.6% (Lang Son) of the samples found positive. 20% of the samples from Tuyen Quang and Thai Nguyen were found with the contamination. In autumn, contamination of *Staphylococcus aureus* on beef sharply decreased, especially in winter in comparison with the rates in spring ($P < 0.05$), with only 3.3% (Bac Giang, Tuyen Quang, Thai Nguyen) to 6.6% (Lang Son) of the samples found positive.

Staphylococcus aureus found on the beef samples in spring was 36.6% (Tuyen Quang) to 56.6% (Thai Nguyen); 43.3%, of the samples from Lang Son and 50.0% of the samples from Bac Giang was found possitive. In summer, contamination of *Staphylococcus aureus* on beef was slightly lower than the rates in spring ($P > 0.05$) with from 16.6% (Bac Giang) to 26.6% (Lang Son) of the samples found positive. 20% of the samples from Tuyen Quang and Thai Nguyen were found with the contamination. In autumn, contamination of *Staphylococcus aureus* on beef sharply decreased, especially in winter in comparison with the rates in spring ($P < 0.05$), with only 3.3% (Bac Giang, Tuyen Quang, Thai Nguyen) to 6.6% (Lang Son) of the samples found positive.

E. coli found on the beef samples in Spring was 80.0% (Tuyen Quang, Lang Son) to 90,0% (Bac Giang); 83.3% from Thai Nguyen. In summer, contamination of *E. coli* on beef was slightly lower than the rates in spring ($P > 0.05$), with from 60.0% (Thai Nguyen) to 83.3% (Bac Giang), contamination of Lang Son was 76.6%, Tuyen Quang was 66.6%. In autumn, contamination of *E. coli* on beef sharply decreased, especially in Winter in comparison with the rates in spring ($P < 0.05$), with only 26.6% (Tuyen Quang) to 36.6% (Bac Giang and Thai Nguyen), the beef samples of Lang Son, contamination of *E. coli* was 33.3%.

Salmonella spp. on beef in spring was 20.0% (Tuyen Quang) to 30.0% (Bac Giang); samples from Thai Nguyen, contamination was 23.3%, samples from Lang Son was 26.6%. In summer, contamination of *Salmonella* spp. on beef was slightly lower

Table 13: Prevalence of *Listeria monocytogenes, E. coli, Salmonella spp.* and *Staphylococcus aureus* in beef sold at the markets by seasons

Type of bacteria	Market	Seasons											
		Spring (Feb-Apr)			Summer (May-Jul)			Autumn (Aug-Oct)			Winter (Nov-Jan)		
		No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)
Listeria monocytogenes	BG	30	6	20.0	30	4	13.3	30	1	3.3	30	1	3.3
	TQ	30	3	10.0	30	1	3.3	30	1	3.3	30	1	3.3
	LS	30	3	10.0	30	2	6.6	30	1	3.3	30	0	0
	TN	30	13	43.3	30	7	23.3	30	2	6.6	30	2	6.6
Staphylococcus aureus	BG	30	15	50.0	30	5	16.6	30	3	10.0	30	1	3.3
	TQ	30	11	36.6	30	6	20.0	30	2	6.6	30	1	3.3
	LS	30	13	43.3	30	8	26.6	30	3	10.0	30	2	6.6
	TN	30	17	56.6	30	6	20.0	30	2	6.6	30	1	3.3
E. coli	BG	30	27	90.0	30	25	83.3	30	15	50.0	30	11	36.6
	TQ	30	24	80.0	30	20	66.6	30	12	40.0	30	8	26.6
	LS	30	24	80.0	30	23	76.6	30	19	63.3	30	10	33.3
	TN	30	25	83.3	30	18	60.0	30	16	53.3	30	11	36.6
***Salmonella* spp.**	BG	30	9	30.0	30	5	16.6	30	3	10.0	30	1	3.3
	TQ	30	6	20.0	30	3	10.0	30	1	3.3	30	2	6.6
	LS	30	8	26.6	30	3	10.0	30	3	10.0	30	2	6.6
	TN	30	7	23.3	30	3	10.0	30	2	6.6	30	2	6.6

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than the rates in spring (*P > 0.05*), with from 10.0% (Tuyen Quang, Lang Son, Thai Nguyen) to 16.6% (Bac Giang). In autumn, contamination of *Salmonella* spp. on beef sharply decreased, especially in winter in comparison with the rates in spring (*P < 0.05*), with only 3.3% (Bac Giang) to 6.6% (Tuyen Quang, Lang Son, Thai Nguyen).

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in pork by seasons

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in pork sold at markets by seasons (spring, summer, autumn, winter) in Bac Giang (BG), Tuyen Quang (TQ), Lang Son (LS), and Thai Nguyen (TN). The results were presented in table 14.

From table 14 we can see the prevelence of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* on pork sold at the markets varied by seasons similar to that of beef, with higher contamination rates in spring and summer, and with lower contamination rates in autumn and winter in the studied sites (*P < 0.05* we can see that the differences were statistically significant). The details are as follows:

Listeria monocytogenes in pork in spring was 10.0% (Tuyen Quang) to 23.33% (Thai Nguyen), samples from Bac Giang was 16.6%, samples from Lang Son was 26.66%. In summer, contamination of *Listeria monocytogenes* was slightly lower than the rates in spring (*P > 0.05*), with from 6.66% (Tuyen Quang, Bac Giang, Thai Nguyen), to 10.0% (Lang Son). In autumn, contamination of *Listeria monocytogenes* on pork sharply decreased, especially in winter in comparison with the rates in spring (*P < 0.05*), with only 3.33% (Tuyen Quang, Lang Son, Thai Nguyen).

Staphylococcus aureus on pork in spring accounted for 30.0% (Lang Son) to 46.66% (Bac Giang); samples from Tuyen Quang and Thai Nguyen, contamination was 36.6%. In summer, contamination of *Staphylococcus aureus* on pork was slightly lower than the rates in spring (*P > 0.05*), with from 16.66% (LS and TN) to 20.0% (BG and TQ). In autumn, contamination of *Staphylococcus aureus* on pork sharply decreased, especially in winter in comparison with the rates in spring (*P < 0.05*), with only 3.33% (TQ to 6.66% (BG, LS, TN).

E. coli on pork in spring was 80.0% (TQ) to 90.0% (LS); a sample from BG was 86.66%, a sample from TN was 83.33%. In summer, contamination of *E. coli* on pork was slightly lower than the rates in spring (*P > 0.05* was not statistically significant), with from 66.66% (BG, TQ) to 83.33% (LS). In autumn, contamination of of *E. coli* on pork sharply decreased, especially in winter in comparison with the rates in spring (*P < 0.05*), with only 20.0% (TQ) to 33.33% (TN); samples from BG was *E. coli* was 26.66%, samples from LS was 30.0%.

Salmonella spp. in pork in spring was 10.0% (Tuyen Quang) to 26.66% (Bac Giang); from Thai Nguyen, contaminated samples accounted for 20.0%, from Lang Son, contaminated samples accounted for 13.33%. In summer, contamination of *Salmonella* spp. on pork was slightly lower than the rates in spring (*P >*

Table 14: Prevalence of *Listeria monocytogenes, E. coli, Salmonella spp. and Staphylococcus aureus* in pork sold at the markets by seasons

Type of bacteria	Market	Seasons											
		Spring (Feb-Apr)			Summer (May-Jul)			Autumn (Aug-Oct)			Winter (Nov-Jan)		
		No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)
Listeria monocytogenes	BG	30	5	16.66	30	2	6.66	30	1	3.33	30	0	0
	TQ	30	3	10.0	30	2	6.66	30	0	0	30	1	3.33
	LS	30	8	26.66	30	3	10.0	30	2	6.66	30	1	3.33
	TN	30	7	23.33	30	2	6.66	30	2	6.66	30	1	3.33
Staphylococcus aureus	BG	30	14	46.66	30	6	20.0	30	2	6.66	30	2	6.66
	TQ	30	11	36.66	30	6	20.0	30	2	6.66	30	1	3.33
	LS	30	9	30.0	30	5	16.66	30	2	6.66	30	2	6.66
	TN	30	11	36.66	30	5	16.66	30	2	6.66	30	2	6.66
E. coli	BG	30	26	86.66	30	20	66.66	30	14	46.66	30	8	26.66
	TQ	30	24	80.0	30	20	66.66	30	10	33.33	30	6	20.0
	LS	30	27	90.0	30	25	83.33	30	15	50.0	30	9	30.0
	TN	30	25	83.33	30	23	76.66	30	18	60.0	30	10	33.33
***Salmonella* spp.**	BG	30	8	26.66	30	4	13.33	30	2	6.66	30	0	0
	TQ	30	3	10.0	30	2	6.66	30	1	3.33	30	0	0
	LS	30	4	13.33	30	2	6.66	30	1	3.33	30	1	3.33
	TN	30	6	20.0	30	3	10.0	30	2	6.66	30	1	3.33

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0.05), with from 6.66% (Tuyen Quang, Lang Son) to 13.33% (Bac Giang); from Thai Nguyen, contaminated samples accounted for 10.0%. In autumn, contamination of *Salmonella* spp. on pork sharply decreased, especially in winter in comparison with the rates in spring (*P < 0.05*), with only 3.33% (LS and TN); None of the samples from BG and TQ were found positive.

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in poultry by seasons

Detection of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* in poultry sold at markets by seasons (spring, summer, autumn, winter) was carried out. The results were presented in table 15.

From table 15 we can see the prevalence of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* on poultry sold at the markets varied by seasons similar to that of beef, with higher contamination rates in spring and summer, and with lower contamination rates in autumn and winter in the studied sites (with *P < 0.05* we can see that the differences were statistically significant). The details are as follows:

Listeria monocytogenes on poultry in spring accounted for 10.0% (LS) to 16.66% (TN), the rate of contaminated samples from BG and TQ was 13.33%. In summer, contamination of *Listeria monocytogenes* was slightly lower than the rates in spring (*P > 0.05*), with 6.66% (Tuyen Quang, Bac Giang, and Thai Nguyen). In autumn, contamination of *Listeria monocytogenes* on poultry sharply decreased, especially in winter in comparison with the rates in Spring (*P < 0.05*), with only 3.33% (BG, TN); None of the samples from LS and TQ was found positive.

Staphylococcus aureus on poultry in spring accounted for 23.33% (Lang Son) to 30.0% (Bac Giang); samples from Tuyen Quang and Thai Nguyen, the contaminated samples accounted for 26.66%. In summer, contamination of *Staphylococcus aureus* on poultry was slightly lower than the rates in spring (*P > 0.05*), with from 10.0% (TQ) to 15.66% (BG); the contaminated samples from LS and TN accounted for 13.33%. In autumn, contamination of *Staphylococcus aureus* on poultry sharply decreased, especially in winter in comparison with the rates in Spring (*P < 0.05*), with only 3.33% (LS) to 6.66% (BG, TQ, TN).

E. coli on poultry in spring accounted for 60.0% (TQ) to 66.66% (BG, LS, TN). In summer, contamination of *E. coli* on poultry was slightly lower than the rates in spring (*P > 0.05* was not statistically significant), with from 26.66% TN to 56.66% (BG); for samples from LS, the rate was 30.0%, and for samples from TQ, the contamination rate was 36.6%. In autumn, contamination of *E. coli* on poultry sharply decreased, especially in winter in comparison with the rates in Spring (*P < 0.05*), with only 10.0% (TN and BG) to 13.33% (LS and TQ).

Salmonella spp. on poultry in spring accounted for 16.6% (TN) to 33.33% (Bac Giang); in LS, the contaminated samples accounted for 23.33%, in TQ, the contaminated samples accounted for 20.0%. In summer, contamination of *Salmonella* spp. on poultry was slightly lower than the rates in spring (*P > 0.05*), with from 6.66% (TN) to 20.0% (Bac Giang); in TQ and LS, the contaminated samples accounted for 10.0%. In autumn, contamination of *Salmonella* spp. on poultry sharply decreased, especially in winter in comparison with the rates in spring (*P < 0.05*), with only 3.33% (TQ) to 6.66% (BG); None of the samples

Table 15: Prevalence of *Listeria monocytogenes, E. coli, Salmonella spp. and Staphylococcus aureus* in poultry sold at the markets by seasons

Type of bacteria	Market	Seasons											
		Spring (Feb-Apr)			Summer (May-Jul)			Autumn (Aug-Oct)			Winter (Nov-Jan)		
		No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)	No. of samples tested	No. of possitive samples	Rate (%)
Listeria monocytogenes	BG	30	4	13.33	30	2	6.66	30	1	3.33	30	1	3.33
	TQ	30	4	13.33	30	2	6.66	30	2	6.66	30	0	0
	LS	30	3	10.0	30	2	6.66	30	1	3.33	30	0	0
	TN	30	5	16.66	30	2	6.66	30	2	6.66	30	1	3.33
Staphylococcus aureus	BG	30	9	30.0	30	5	15.66	30	2	6.66	30	2	6.66
	TQ	30	8	26.66	30	3	10.0	30	3	10.0	30	2	6.66
	LS	30	7	23.33	30	4	13.33	30	2	6.66	30	1	3.33
	TN	30	8	26.66	30	4	13.33	30	2	6.66	30	2	6.66
E. coli	BG	30	20	66.66	30	17	56.66	30	12	40.0	30	3	10.0
	TQ	30	20	66.66	30	11	36.66	30	7	23.33	30	4	13.33
	LS	30	18	60.0	30	9	30.0	30	5	16.66	30	4	13.33
	TN	30	20	66.66	30	8	26.66	30	5	16.66	30	3	10.0
***Salmonella* spp.**	BG	30	10	33.33	30	6	20.0	30	2	6.66	30	2	6.66
	TQ	30	6	20.0	30	3	10.0	30	2	6.66	30	1	3.33
	LS	30	7	23.33	30	3	10.0	30	2	6.66	30	0	0
	TN	30	5	16.66	30	2	6.66	30	1	3.33	30	0	0

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from LS and TN was found positive. The results were in line with those on *Salmonella* spp. on chicken meat [18].

Examination for biological and chemical characteristics of the bacteria strains of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* isolates

Examination for biological and chemical characteristics of the bacteria strains of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* isolated (30 bacteria strains on the beef, pork, and poultry samples which did not meet hygiene standard). The results were presented in table 16.

From the results presented in table 16 we can see that: The bacteria strains of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* isolated carried their genus and species typical biological and chemical characteristics as described by Quinn PJ, et al. [35]. The *Listeria monocytogenes* bacteria were found with the following characteristics: Gram positive stain, mobility, catalase, production of listeriolysin, rhamnose fermentation, and no mannitol fermentation (86.6%); The *E. coli* bacteria were found with the following characteristics: Gram negative stain, mobility, causing hemolysis on blood agar (63.3%), lactose fermentation, indole production at 44°C; The *Salmonella* spp. Bacteria develop well in Rappaport-Vassiliadis environment at 42°C, Gram negative stain, mobility (36.6%), khong lactose fermentation, and production of H2S; The *Staphylococcus aureus* bacteria were found with following characteristics: developed well in Chapman Stone Agar environment, produce yellow S (Smooth) colonies, Gram positive stain, immobility, catalase production, coagulase causing blood plasma clotting, and hemolysis (93.3%), sucrose fermentation, and reduction of nitrate into nitrite ($N0_3$ – $N0_2$).

Table 16: Biological and chemical characteristics of *Listeria monocytogenes, E. coli, Salmonella spp.* and *Staphylococcus aureus* isolates

Tests on biological characteristics	Results		
	Strains tested	Reaction	Rate (%)
Listeria monocytogenes			
Gram positive stain	30	30	100
Mobility	30	30	100
Mannitol fermentation	30	0	0
Rhamnose fermentation	30	30	100
Listeriolysin production	30	26	86.6
Catalase production	30	30	100
E. coli			
Gram negative stain	30	30	100
Mobility	30	30	100
Hemolysis on blood agar	30	19	63.3
Lactose fermentation	30	30	100
Production of Indole at 44°C	30	30	100
***Salmonella* spp.**			
Development in Rappaport-Vassiliadis at 42°C	30	30	100
Gram negative stain	30	30	100
Mobility	30	11	36.6
Hemolysis on blood agar	30	0	0
Lactose fermentation	30	0	0
H2S production	30	30	100
Staphylococcus aureus			
Development in Chapman Stone Agar environment	30	30	100
Gram positive stain	30	30	100
Mobility	30	0	0
Catalase reaction	30	30	100
Hemolysis	30	28	93.3
Sucrose fermentation	30	30	100
Coagulase production	30	30	100
Nitrate reduction reaction	30	30	100

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Virulence of the bacteria strains of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* isolated

In order to determine the virulence of the isolates, tests on virulence of the bacteria strains of *E. coli, Salmonella* spp. and *Listeria monocytogenes* on pork sold at the markets (selected from the samples which were found positive samples did not meet hygiene standard) with typical biological and chemical characteristics were performed on laboratory mice. The results are presented in table 17.

From table 17 we can see that: After being infected with the bacteria on meat, the tested mice died within 8h to 48h. Specifically: *Listeria monocytogenes* caused death for 93.3% of the tested mice; *E. coli* caused deaths for 91.6%, *Salmonella* spp. cause deaths for 95.0%; *Staphylococcus aureus* caused deaths for 93.3%. After being infected with the bacteria, the tested mice showed symptoms including ceasing eating and decreasing of mobility. Operation on dead mice revealed that their colon was full of air, their spreen was swollen, their liver was bleeding and there was a fluid in the thoracic and abdominal cavities... The bacteria were reisolated from the dead tested mice with the rate of 100%. The result showed the seriousness of food poison causing ability of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* on meat as described in the studies of Quinn PJ, et al. [35].

Table 17: Virulence of *Listeria monocytogenes, E. coli, Salmonella spp.* and *Staphylococcus aureus* isolated

Types of bacteria	Number of strains	Number of tested mice	Dose of abdominal injection (ml/mouse)	Number dead mouse after being infected					Mortality rate (%)
				8 hours	24 hours	32 hours	48 hours	6 days	
Listeria monocytogenes	30	60	0.2	18	23	12	3	0	93.3
E. coli	30	60	0.2	12	26	14	3	0	91.6
***Salmonella* spp.**	30	60	0.2	19	28	8	2	0	95.0
Staphylococcus aureus	30	60	0.2	19	28	7	2	0	93.3

Determination of the serotype of *E. coli* strains

Serotype O of the *E. coli* strains isolated on meat with levels of contamination surpassing hygiene standards, the results are presented in table 18.

From table 18 we can see that 11 *E. coli* strains isolated belonged to serotype O!57:H7 (accounting for 2.95%); 56 strains belonged to O26 and O111 (accounting for 15.05%); 43 strains belonged to O55 (accounting for 11.55%); 35 strains belonged to O103 (accounting for 9.40%); 32 strains belonged to O121 (accounting for 8.60%); 29 strains belonged to O138 and O139 (accounting for 7.79%); 26 strains belonged to O145 (accounting for 6.98%); 55 strains belonged to other serotype O (accounting for 14.78%). The results found on *E. coli* O157:H7 were in line with that of contamination of 1.7% on shop equipment, 2% on the meat on sale, and 3.3% on the chopping boards [7]. The result confirmed the virulence of *E. coli* and other food poison causing bacteria on food poisoning.

Table 18: Distribution of antigen serotype O of *E. coli* strains on meat

Sources	No. of strains	Results									
		O157:H7	O26	O55	O103	O111	O121	O138	O139	O145	O Khác
Beef	130	4	29	12	8	21	12	10	9	11	14
Pork	176	5	21	28	19	23	18	16	15	12	19
Poultry	66	2	6	3	8	12	2	3	5	3	22
Total	**372**	**11**	**56**	**43**	**35**	**56**	**32**	**29**	**29**	**26**	**55**

Determination of serotype of *Salmonella* spp. strains

Determination of serotype of *Salmonella* spp. strains isolated were performed on the beef, pork, and poultry samples, the results were presented in table 19.

From table 19 we can see that the 152 *Salmonella* spp. Bacteria isolated consisted of the following serotypes: 15 strains of *S. typhimurium* on meat (9.8%), 18 strains of *S. choleraesuis* (11.8%), 5 strains of *S. enteritidis* (3.2%), 5 strains of *S. weltevreden* (3.2%); and 3 strains of *S. anatum* (1.9%) [1,13,18].

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Table 19: Serotype of *Salmonella* spp. strains isolated

Sources	No. of strains tested	O	H1	H2	S.typhimurium	S.choleraesuis	S.enteritidis	S.weltevreden	S. anatum
Beef	60	1, 4, 5, 12 10 1, 9	i r g,m	1,2 z6 -	2 4	6	3	0	1
Pork	40	1,4 3	i r	1,2 z6	5 1	4 2	1	2	0
Poultry	52	1, 4, 5 3, 10	i r	1,2 z6	3	6	1	2 1	2
Total	152				15	18	5	5	3

Determination of the encoded gene producing Staphylococcal enterotoxin B (SEB) of *Staphylococcus* isolates

By using PCR reaction, 146 strains of *Staphylococcus aureus* on meat was examined to determine the encoded producing Staphylococcal Enterotoxin B (SEB) with the specific primers P-SEB-F and P-SEB-R. The results are presented in table 20.

From table 20 we can see that 116 strains of *Staphylococcus aureus* possessed the DNA with the encoded gene producing SEB (79.4%);30 strains did not possess the gene (20.5%). The results were in line with those in the studies on *Staphylococcus aureus* on meat and the results on determination of SEB produced by this type of bacteria of [11,22,23].

Table 20: Frequency of the encoded gene producing Staphylococcal enterotoxin B (SEB) of *Staphylococcus aureus* isolates

Sources of *Staphylococcus aureus* isolates	No. of strains tested	Results			
		No. of *Staphylococcus aureus* strains possessing the gene	Rate (%)	No. of *Staphylococcus aureus* strains not possessing the gene	Rate (%)
Beef	62	46	74.1	16	25.8
Pork	46	39	84.7	7	15.2
Poultry	38	31	81.5	7	18.4
Total	146	116	79.4	30	20.5

Detection of verotoxins VT1 and VT2 produced by *E. coli* on meat

PCR reactions were conducted for detecting the DNA carrying the encoded gene producing VT1 and VT2 of serotype *E. coli* on meat. The results are presented in table 21.

From table 21 we can see that out of the 372 *E. coli* strains examined, it was found that 44 carried VT1, accounting for 11.82%; of which 2 strains belonged to serotype O157:H7 (18.18%); 6 strains belonged to O26 (10.71%); 6 strains belonged to O55 (13.95%)..., with the highest rates of the strains belonging to serotypes O 145 (23.07%) and O138 (24.13%); 35 of the strains were found carrying gene VT2, accounting for 9.40%; of which 3 strains belonged to serotype O157:H7 (27.27%); 5 strains belonged to O103, and 8 strains belonged to O111 (14.28%); 5 strains belonged to O139 (17.24%); 1 strain belonged to O145 (3.84%), with the lowest rates of the strains belonging to O121 ((0%) and O26 (3.57%). There were 12 strains carrying both VT1 and VT2, accounting for 3.22%.

Thus, the *E. coli* strains isolated possessed the encoded virulence genes VT1 and VT2 belonged to the serotypes O157:H7, O26, O111, O55, O138, O139, O145, and other serotype O (serotype O not identified). The results were in line with those on the presence of the encoded virulence genes VT1 and VT2 of *E. coli* isolated from the meat shops in Ontario, Canada [26,32] (Figure 1).

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Table 21: Frequency of appearance of the encoded gene producing VT1 and VT2 by *E. coli* on

Serotype *E. coli*	No. of strains examined	Frequency of appearance					
		VT1		VT2		VT1+VT2	
		No. of strains found with the gene	Rate %	No. of strains found with the gene	Rate %	No. of strains found with the gene	Rate %
O157:H7	11	2	18.18	3	27.27	1	9.09
O26	56	6	10.71	2	3.57	2	3.57
O55	43	6	13.95	3	6.97	1	2.32
O103	35	0	0	5	14.28	0	0
O111	56	2	3.57	8	14.28	1	1.78
O121	32	5	15.62	0	0	0	0
O138	29	7	24.13	4	13.79	2	6.89
O139	29	5	17.24	5	17.24	1	3.44
O145	26	6	23.07	1	3.84	2	7.69
Other O	55	5	9.09	4	7.27	2	3.63
Total	**372**	**44**	**11.82**	**35**	**9.40**	**12**	**3.22**

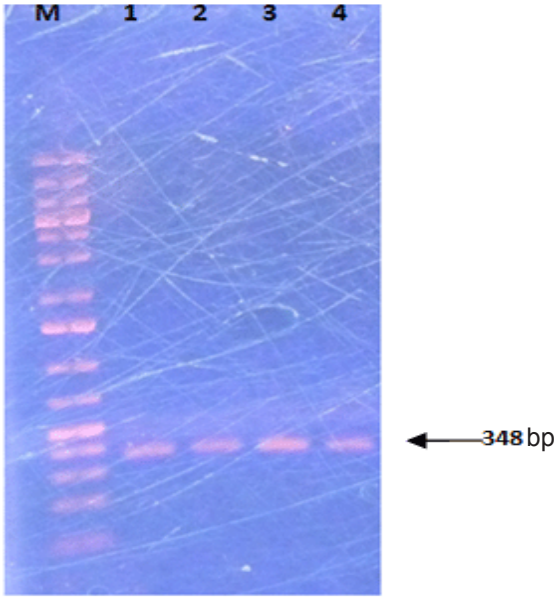

Figure 1: Agarose gel electrophoresis of PCR amplification products using specific Verotoxin gene (VT primers) of *E. coli* isolated. Lane M:100 bp ladder as molecular DNA marker, Lane 1: Control positive, Lane 2, Lane 3 and lane 4: Positive *E. coli* for Verotoxin production

Detection of encoded gene producing *Stn, InvA* of *Salmonella* spp. isolates

PCR reactions were performed to detect DNA possessing the encoded gene producing enterotoxin *Stn* and invasive abililty *InvA* of *Salmonella* spp. (*S. typhimurium*, *S. choleraesuis, S. enteritidis, S. weltevereden, S anatum*) strains on meat. The results are presented in table 22.

Table 22 shows that the *Salmonella* spp. bacteria on meat possessed the follwing enterotoxin encoded gene: 86.6% *Stn* (*S. typhimurium*), 66.6% *S. choleraesuis* and *S. anatum*, 100% *S. enteritidis,* and 80.0% *S. weltevreden.* On the invasive ability of the *Salmonella* spp. bacteria strains isolated found with the encoded gene, the results were as follows: 73.3% *S. typhimurium*, 50.0% *S. choleraesuis,* 80.0% *S. enteritidis*, 40.0% *S. weltevreden,* and 66.6% *S. anatum*. The results were similar to those in the studies on production of *Stn* and *InvA* of *Salmonella* enterica on beef, pork, and poultry [3,8]. However, they were lower than those of with 100% on enterotoxin *Stn* production and 100% *invA* invasion [4] (Figure 2).

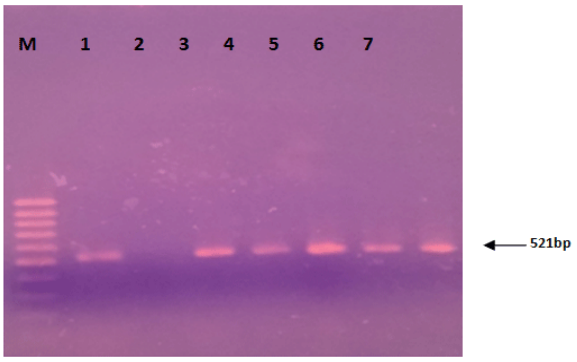

Figure 2: Agarose gel electrophoresis of PCR amplification products using specific Invasion gene (*InvA* primers) of *Salmonella* spp. isolated. Lane M:100 bp ladder as molecular DNA marker, Lane 1: Control positive, Lane 2: Negative *Salmonella* spp. for InvA production, Lane 3, Lane 4, Lane 5, Lane 6 and Lane 7: Positive *Salmonella* spp. for *InvA* production.

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Table 22: Presense of the encoded gene producing *Stn*, *InvA* of *Salmonella* spp. on meat

Salmonella spp.	No. of strains examined	Presence			
		Stn		*InvA*	
		No. of strains found with the encoded gene	Rate %	No. of strains found with the encoded gene	Rate %
S. typhimurium	15	13	86.6	11	73.3
S. choleraesuis	18	12	66.6	9	50.0
S. enteritidis	5	5	100.0	4	80.0
S. weltevreden	5	4	80.0	2	40.0
S. anatum	3	2	66.6	2	66.6
Total	**46**	**36**	**78.2**	**28**	**60.8**

Determination of DNA carrying disease causing encoded gene Listeriolysin (hly A) of *Listeria monocytogenes* with PCR

PCR reactions were performed to identify Listeriolysin O (LLO), the main toxin-producing factor of *Listeria monocytogenes*. It was found that with the bacteria strains of *Listeria monocytogenes* isolates, the DNA carrying the encoded gene hlyA produced hemolysis causing Listeriolysin O. The results are presented in table 23.

Table 23 presented the results of determination of the number of strains possessing the encoded gene LLO (hlyA) of *Listeria monocytogenes* isolates; It was found that with *Listeria monocytogenes* on beef, 7/32 strains possessed gene hlyA, accounting for 21.8%; on pork, there were 9/40 strains, accounting for 22.5%; on chicken meat, there were 11/32 strains, accounting for 34.3% [20,25,38] (Figure 3).

Table 23: Determination of the ADN carrying the encoded gene producing Listeriolysin O of *Listeria monocytogenes* isolates

Sources of isolation	No. of strains examined	No. of strains showing DNA with the encoded gene *hlyA* producing LLO	Rate (%)
Beef	32	7	21.8
Pork	40	9	22.5
Poultry	32	11	34.3
Total	**104**	**27**	**25.9**

Susceptibility to antibiotics and medicinal chemicals of *Listeria monocytogenes, E. coli, Salmonella* spp. and *Staphylococcus aureus* isolates

Tests were carried out to determine susceptibility to antibiotics and medicinal chemicals of the 27 strains of *Listeria monocytogenes* (strains carrying virulence gene hlyA), 116 strains of *Staphylococcus aureus* (strains carrying gene producing Staphylococcal enterotoxin B), 79 strains of *E. coli* (strains carrying virulence gene VT1 and VT2), and 46 strains of *Salmonella* spp. (strains carrying virulence gene *Stn* and *InvA*) isolates. The results are presented in table 24.

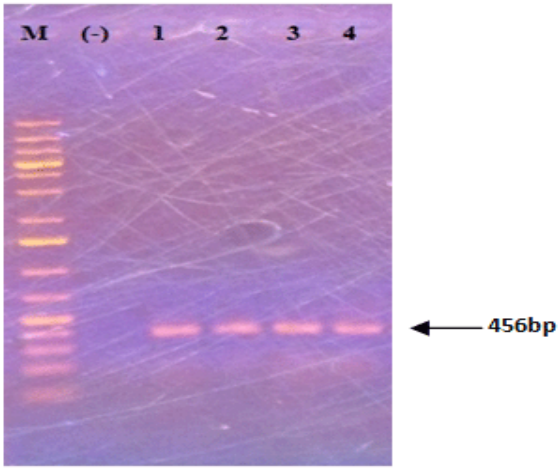

Figure 3: Agarose gel electrophoresis of PCR amplification products using specific listeriolysin gene (hlyA primers) of L. monocytogenes isolated. Lane M: 100 bp ladder as molecular DNA marker, Lane (-): Negative L. monocytogenes for listeriolysin production, Lane 1: Control positive, Lane 2, Lane 3 and lane 4: Positive L. monocytogenes for listeriolysin production.

Table 24 showed antimicrobial resistance of *Listeria monocytogenes*, Staphylococcus aureus, *E. coli*, and *Salmonella* spp. isolates. The details are as follows:

There were 21/27 strains of *Listeria monocytogenes* resisting to amoxicilline, accounting for 77.77%; 1/27 strains resisting to nitrofurantoin, ceftazidime, oxytetracycline, accounting for 3.70%; 3/27 strains resisting to erythromycin, accounting for 11.11%; None of the strains (0%) were resistant to vancomycin and oxacillin; 2/27 strains resisting to rifampicin, accounting for 7.40%; 5/27 strains resisting to gentamicin, accounting for 18.51%; 3/27 strains resisting to bacitracin, accounting for 11.11%; 4/27 strains resisting to nalidixic acid (14.81%). Ciprofloxacin and kanamycin had no pharmaceutical effect on *Listeria monocytogenes*, which means the rate of antimicrobial resistance was 100%. The results were similar to those in a study on susceptibility to antibiotics of *Listeria monocytogenes* isolated from poultry in Oyo, Southwest Nigeria [19].

There were 96/116 strains of *Staphylococcus aureus* resisting

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Table 24: Antimicrobial resistance of *Listeria monocytogenes, E. coli, Salmonella spp.* and *Staphylococcus aureus* isolates

Antibiotics and Medicinal Chemicals	Antimicrobial resistance of the bacteria isolates											
	Listeria monocytogenes			*Staphylococcus aureus*			*E. coli*			*Salmonella* spp.		
	No. of strains tested	No. of strains found with antimicrobial resistance	Rate (%)	No. of strains tested	No. of strains found with antimicrobial resistance	Rate (%)	No. of strains tested	No. of strains found with antimicrobial resistance	Rate (%)	No. of strains tested	No. of strains found with antimicrobial resistance	Rate (%)
Amoxicilline	27	21	77.77	116	96	82.75	79	79	100	46	46	100
Nitrofurantoin	27	1	3.70	116	5	4.31	79	2	2.53	46	1	2.17
Ciprofloxacin	27	27	100	116	116	100	79	5	6.32	46	3	6.52
Erythromycin	27	3	11.11	116	18	15.51	79	12	15.18	46	9	19.56
Ceftazidime	27	1	3.70	116	4	3.44	79	3	3.79	46	3	6.52
Vancomycin	27	0	0	116	0	0	79	79	100	46	46	100
Kanamycin	27	27	100	116	116	100	79	6	7.59	46	3	6.52
Rifampicin	27	2	7.40	116	3	2.58	79	5	6.32	46	4	8.69
Gentamicin	27	5	18.51	116	62	53.44	79	16	20.25	46	11	23.91
Bacitracin	27	3	11.11	116	8	6.89	79	38	48.10	46	29	63.04
Oxacillin	27	0	0	116	2	1.72	79	79	100	46	46	100
Nalidixic acid	27	4	14.81	116	32	27.58	79	0	0	46	0	0
Oxytetracycline	27	1	3.70	116	3	2.58	79	1	1.26	46	0	0

to amoxicilline (82.75%); 5/116 strains resisting to nitrofurantoin (4.31%); 18/116 strains resisting to erythromycin (15.51%); 4/116 strains resisting to ceftazidime (3.44%); None of the strains were found resisting to vacomycin (0%); 3/116 strains resisting to rifampicin and oxytetracycline (2.58%); 62/116 strains resisting to gentamicin (53.44%); 8/116 strains resisting to bacitracin (6.89%); 2/116 strains resisting to oxacillin (1.72%); 32/116 strains resisting to nalidixic acid (27.58%). Ciprofloxacin and kanamycin had no phamaceutical effect on Staphylococcus aureus, which means the rate of antimicrobial resistance was 100%. The results were in line with those on antimicrobial resistance of *Staphylococcus aureus* on food in China [43].

There were 2/79 strains of *E. coli* strains resisting to nitrofurantoin (2.53%); 5/79 strains resisting to ciprofloxacin (6.32%); 12/79 strains resisting to erythromycin (15.18%); 3/79 strains resisting to ceftazidime (3.79%); 6/79 strains resisting to kanamycin (7.59%); 5/79 strains resisting to rifampicin (6.32%); 16/79 strains resisting to gentamicin (20.25%); 38/79 strains resisting to bacitracin (48.10%); None of the strains were found resisting to nalidixic acid (0%); 1/79 strains resisting to oxytetracycline (1.26%). Amoxicilline, vancomycin, and oxacillin had no phamaceutical effect on *E. coli*, which means the rate of antimicrobial resistance was 100%. The results were in line with those on antimicrobial resistance of *E. coli* on beef in Onrario, Canada [26].

There were 1/46 strains of *Salmonella* spp. resisting to nitrofurantoin (2.17%); 3/46 strains resisting to ciprofloxacin, kanamycin and ceftazidime (6.52%); 9/46 strains resisting to erythromycin (19.56%); 4/46 strains resisting to rifampicin (8.69%); 11/46 strains resisting to gentamicin (23.91%); 29/46 strains resisting to bacitracin (63.04%); None of the strains were found resisting to nalidixic acid and oxytetracycline (0%). Amoxicilline, vancomycin and oxacillin had no phamaceutical effect on *E. coli*, which means the rate of antimicrobial resistance was 100%. The results were in line with those on susceptibility to antibiotics of *Salmonella* spp. on meat isolated [10].

Discussion

Proportion of the beef, pork, and poultry sold at the markets in Bac Giang, Tuyen Quang, Lang Son and Thai Nguyen under slaughter control was small, accounting for only 8% to 14%; for the rest, it was unable to trace the sources of the meat; the scattered and small slaughtering points where the cattle's and poultry were killed did not meet hygiene standard. The results reflect the current situation of cattle and poultry slaughtering and meat selling in Vietnam, there were more than 29,840 small slaughter houses with the capacity of 1- 3 cattle or poultry per day, with nearly 22,000 were not under the control of local department of animal health [9].

The meat samples collected at the markets did not meat hygiene requirements, with high rates of aerobe bacteria contaminated (46.6% of the beef samples, 48.3% of the pork samples, and 36.6% of the poultry samples). The result of the survey on enumeration of total aerobic bacteria on the meat samples showed us the hygienic quality of the meat sold in the markets. The results were in line with those on total aerobic bacteria on meat; on the number of bacteria found on chicken

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meat; and on micro bacteria on beef in Kigali city, Rwanda [33,17,12].

Contamination in beef of *Listeria monocytogenes* was 10%, *Staphylococcus aureus* 21.6%; *E. coli* 65.0%, and *Salmonella* spp. was 15.0%; in pork, the highest contamination rate with *Listeria monocytogenes* was 11.6% with *Staphylococcus aureus* was 20.0%; with *E. coli* was 63.3%; and with *Salmonella* spp. was 11.6%; in poultry, the highest contamination rate with *Listeria monocytogenes* was 8.3%; with *Staphylococcus aureus* 15.0%; with *E. coli* 43.3%, and with *Salmonella* spp. was 10.0%. The figures were lower than those of with *Listeria monocytogenes* on beef accounted for 47.7%; in line with those in Saudi Arabia and Egypt with 12% to 38% of the beef samples found contaminated with *Staphylococcus aureus*); with those with *Escherichia coli* isolated on pork 67.7%, beef 27.7%, used water 2.3% and from human 0.77%); and in line with those with *Salmonella typhimurium* found on beef was 4.6% [37,24, 5,1].

Contamination of *Listeria monocytogenes*, *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* in beef, pork, and poultry varied by sample collection time during the day in studied sites ($P < 0.05$). The results were lower than those on *Listeria monocytogenes* contamination, with 40% of the samples found positive on poultry, red meat and meat products; and with 47.7% of the beef samples found positive with *Listeria monocytogenes*; and were in line with those in Saudi Arabia and Egypt with 12% to 38% of the beef samples found positive with *Staphylococcus aureus;* in line with those, who successfully isolated Escherichia coli from beef (27.7%) and used water (2.3%); with those with 4.6% of the beef samples found positive with *Salmonella typhimurium* of [20,24,37,5,1].

One of the specific objectives of this study is to determine the relation between the consumption of the beef, pork, and poultry at the markets and level of contamination of *Listeria monocytogenes*, *Staphylococcus aureus, E. coli*, and *Salmonella* spp in the meats. Thus, we collected random samples at different time frames during the day (from 6h to 11h, from 14h to 16h, and from 17h to 19h). Besides, collecting the samples at different time frame like this also makes it possible to evaluate the influence of the time that the meat is kept the shelves in the markets on the level of contamination of the bacteria.

It was observed that the variation of the results among sample collecting time frames related to unhygienic meat selling conditions. After contaminating the meat, the food poisoning bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli*, and *Salmonella* spp.) continued to reproduce. The duration of meat exposure for sale in the markets and the temperature conditions were favorable for the bacteria to multiply.

Contamination of *Listeria monocytogenes*, *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* in fresh meat sold at the markets varied by seasons with higher contamination rates in spring and summer, and with lower contamination rates in autumn and winter in the studied sites (with $P < 0.05$). The results were in line with those on contamination of *Listeria monocytogenes* on meat samples collected in 24 cities in China, with 5.4% to 37.8% of the samples contaminated [43].

Our additional discussion: Both weather conditions (winter and summer time), and exposure for sale at the markets are influential factors to the level of contamination of the bacteria (*Listeria monocytogenes*, *Staphylococcus aureus, E. coli,* and *Salmonella* spp.) on the meat. This is in line with the increase of the number of cases of food poisoning in winter and summer, and the decrease of that in autumn and winter.

At the studied sites, the meat was carried whole piece from slaughter house to the markets. The meat was exposed for sale from 6:00 to 19:00 in natural conditions, without being kept in cool boxes or cold storehouses, which created favorable conditions for the bacteria to grow and reproduce. These were the main limitations of the storage conditions which led to the contamination of food poisoning bacteria including *Listeria monocytogenes*, *Staphylococcus aureus, E. coli*, and *Salmonella* spp. in meat with the high level of contamination and high count of bacteria as found in this study, and as warned [14].

Listeria monocytogenes, *E.coli*, *Salmonella* spp. and *Staphylococcus aureus* isolated carried typical biological and chemical characteristics of their genus and species; had disease-causing ability (pathogenicity), with high virulence on tested mice, causing deaths to 91.6% to 95.0% of the tested mice within 48 hours after being infected with the bacteria; *E. coli* isolated belonged to serotype O157:H7 (2.95%); O26 and O111 (15.05%); O55 (11.5%); O103 (9.4%); O121 (8.6%); O138 and O139 (7.7%); O145 (6.9%); other serotype O accounted for 14.7%, carrying the encoded gene producing VT1 accounted for 11.8%; VT2 accounted for 9.4%, VT1 + VT2 accounted for 3.2%; *Salmonella* spp. isolated belonged to serotype *S. typhimurium* (9.8%); *S. choleraesuis* (11.8%); *S. enteritidis* (3.2%); *S. weltevreden* (3.2%); and *S. anatum* (1.9%); *Staphylococcus aureus* had DNA carrying the encoded gene producing SEB (79.4%); 30 strains did not carry the gene (20.5%); *Salmonella* spp. on meat carried the encoded gene producing enterotoxin Stn accounted for 78,2%, InvA accounted for 60,8%; *Listeria monocytogenes* on beef carrying hlyA accounted for 21.8%, pork accounted for 22.5%, poultry accounted for 34.3%.

Listeria monocytogenes resisting to amoxicilline accounted for 77.7%; resisting to nitrofurantoin, ceftazidime, oxytetracycline accounted for 3.7%; were resistant to erythromycin, with 11.1%; were resistant to lại rifampicin, with 7.4%; were resistant to gentamicin, with 18.5%; were resistant to bacitracin, with 11.1%; and were resistant to nalidixic acid (14.8%).

Staphylococcus aureus were resistant to amoxicilline (82.7%); were resistant to nitrofurantoin (4.3%); were resistant to erythromycin (15.5%); were resistant to ceftazidime (3.4%); were resistant to rifampicin and oxytetracycline (2.5%); were resistant to gentamicin (53.4%); were resistant to bacitracin (6.8%); were resistant to oxacillin (1.7%); were resistant to Nalidixic acid (27.5%).

E. coli were resistant to nitrofurantoin (2.5%); were resistant to ciprofloxacin (6.3%); were resistant to erythromycin (15.1%);

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were resistant to ceftazidime (3.7%); were resistant to kanamycin (7.5%); were resistant to rifampicin (6.3%); were resistant to gentamicin (20.2%); were resistant to bacitracin (48.1%); and were resistant to oxytetracycline (1.2%).

Salmonella spp. were resistant to nitrofurantoin (2.1%); were resistant to ciprofloxacin, kanamycin and ceftazidime (6.5%); were resistant to erythromycin (19.5%); were resistant to rifampicin (8.6%); were resistant to gentamicin (23,9%); and were resistant to bacitracin (63.0%).

Conclusion

In the North of Vietnam, veterinary techniques and management on food safety are insufficient. Due to the lack of concentrated slaughterhouses for slaughtering the poultry and cattle, the places used for slaughtering them are not the ones that have been approved by the authority. The controlling of veterinary hygiene in general and the monitoring of veterinary hygiene in the markets in particular are mainly superficial and do not meet the technical requirements.

The limitations in food safety and veterinary hygiene techniques and management lead to the high level of contamination of aerobe in beef, pork and poultry meat sold at the markets, including *Listeria monocytogenes*, *E. coli*, *Salmonella* spp. and *Staphylococcus aureus.*

The bacteria isolated bear toxin producing genes with high virulence which can cause diseases and food poisoning. The *Listeria monocytogenes*, *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* bacteria are resistant to common antibiotics such as amoxicilline, nitrofurantoin, ceftazidime, ciprofloxacin, kanamycin, rifampicin, and erythromycin.

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