

## Prevalence of *Salmonella* spp. in chicken and beef from retail outlets in Malaysia

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### Abstract

The prevalence of *Salmonella* in chicken and beef sold in retail outlets in Malaysia was determined by analysing 312 raw beef and chicken meat samples including their processed products. Samples purchased from supermarkets, butcher shops and wet market, which being classified into raw, minced and processed chicken and beef. A total of 86 (27.6%) samples were found positive for *Salmonella* spp., with chicken meat samples (40.4%) showed greater presence compared to beef (15.4%). Highest presence of *Salmonella* were detected from wet market samples (35.4%), followed by supermarket (26.9%) and butcher shop (21.3%). The prevalence of *Salmonella* were higher in unpacked chicken meat (84.8%), followed by unpacked beef (27.8%). *Salmonella* serovars were identified as *S. Enteritidis*, *S. Hadar*, *S. Dublin*, *S. Anatum*, *S. Stanley*, *S. Gallinarum*, *S. Choleraesuis* and *S. Typhimurium*. Detection of 8 *Salmonella* serovars showed possibilities of cross contamination in various sources either at slaughtering house, processing plant or until storage at retail level. Improper cooking method on meats and hygiene practices prior to consume should be avoided in order to ensure food safety before ingestion.

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### Keywords

*Salmonella*  
Retail  
Beef  
Chicken  
Package

### Introduction

*Salmonella* remains significantly high as food borne pathogens compared to others and has not been declined for over a decade. *Salmonella* poses as a leading cause of foodborne diseases in few countries, sometimes contributing to the highest morbidity and mortality rates among foodborne pathogens (CDC, 2009). Most of the *Salmonella* spp. associated to humans and other mammals' diseases are from *S. enterica* subsp. *enterica* with *S. Enteritidis* and *S. Typhimurium* were responsible for most of the infections (Dunkley *et al.*, 2009). Other *Salmonella* serovars are zoonotic or potentially zoonotic, while some usually found in the environment such as *Salmonella bongori*, *Salmonella enterica* subsp. *salamae*, *Salmonella enterica* subsp. *arizonae*, *Salmonella enterica* subsp. *diarizonae*, *Salmonella enterica* subsp. *houtenae* and *Salmonella enterica* subsp. *indica* are occasionally associated with human disease (WHO, 2003; Matheson *et al.*, 2010; Das *et*

*al.*, 2012).

Various food products have been depicted as transporting agent against infection by *Salmonella* to humans, including beef, poultry, pork, eggs and seafood. *Salmonella* is also persistent pathogen that capable of surviving and proliferating in a variety of environmental conditions including food production and processing plant (Mezal *et al.*, 2013). According to El-Aziz (2013), *Salmonella* contamination in beef and chicken can occur at several stages along food supply chain includes productions, processing, distribution, retailing and also preparing and handling by consumers. The persistence of *Salmonella* in premises may include resistance of the specific strains to the disinfection, desiccation and also biofilm production (Vestby *et al.*, 2009).

Foodborne illness worldwide is often related with the consumption of meat and poultry products contaminated with pathogenic microorganism mainly from retail level (Vindigni *et al.*, 2007). The microbiology quality of chicken and beef sold

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may differ based on type of retail outlets depends on the hygiene management, management of the meat supplied from receiving until selling include temperature controls, storage, packing and handling at the point of sale which sometimes involve cutting and mincing. Uncontrolled conditions at the retail level might contribute to the multiplication of the microorganism includes pathogenies especially *Salmonella* spp. where could enhance the risk of contamination at consumer level.

Commonly chicken meat is popular poultry species in the world and considered as the main protein source in Malaysian diet. It is also served as major option of meat offered in most of food service outlets. Due to its popularity, Jayaraman *et al.* (2013) had reported per capita consumption of chicken meat and chicken based products by Malaysian were increased 0.03 million tonnes from 1.4 million tonnes at year 2013 to year 2014. Approximately, 500 million chickens produced by 3,200 broiler grower farms in Peninsular Malaysia which 30% of it went to modern processing plant and sold in supermarket. Remaining sold as live chickens for wet markets (Jayaraman *et al.* 2013; Fadhillah, 2015). To ensure enough supplies, Malaysia also imported chicken most from China then followed by Thailand, Denmark, and the Netherlands (Worldpoultry.net, 2014).

Beside chicken meat, production of beef and beef-based products in Malaysia also increased annually 1.6 million tons reported in 2010 and projected to achieve 2.1 million tons in 2020. Beef products also had increased by around 78% from 29,000 million tons in year 2005 to year 2014. Demand for beef is increasing every year in line with the increase of population and consumption per capita (Fadhillah, 2015).

In Malaysia, both meats are most commonly served in food outlets especially in hotel, restaurants, school canteen and even small outlets which involved various types of preparations. It also being a favourite main dish served during festive and occasions. Thus, these meats are very easily available and sold in all types of retail outlets in Malaysia. The most popular retail outlet visited by Malaysian to get this type of meat supplies are supermarket, butcher shop and wet market which are selected based on their nearest location, cheaper price offered, an adequate supplies and in certain circumstances freshness of the meat and hygiene being a priority criteria. With regards to consumer safety towards the risk of chicken and beef being contaminated with pathogens at the retail level, we conducted this study to determine the prevalence of *Salmonella* spp. in the main chicken and beef sales outlet.

## Materials and Methods

### *Sample collection*

Samples of chicken and beef, including processed products such as minced chicken and minced beef, chicken meatballs and beef balls as well as chicken and beef burgers and frankfurters were purchased from two different types of supermarkets, butcher shops and wet markets located in Selangor and Negeri Sembilan. Same samples were collected in three times from the same outlets to see the *Salmonella* detection in particular outlet. From 312 samples purchased, 72 samples were raw beef, 30 minced beef, 54 processed beef, 72 raw chickens, 30 minced chicken and 54 processed chicken. From these, 108 of the samples were purchased from supermarket, 108 samples from butcher shops and 96 samples from wet market. Aseptic sampling techniques was applied, where the samples were placed in sterile polyethylene bags, kept on ice and sent to laboratory immediately. Analysis was performed within 24 hours after sampling and kept in chilled condition (0°C to 4°C) before pre-treatment.

### *Sample preparation and pre-enrichment*

Different samples preparation were practiced for different types of samples. Standard protocol from United States Department of Agriculture (USDA; MLG 4.08, 2014) was referred for sample preparation and enrichment guide.

### *Raw chicken (chicken parts) and chicken products, including minced chicken meats*

A total of 225±4.5 mL of Buffered Peptone Water (BPW; Oxoid, CM0509B) was added to approximately 25±2.5 g of raw chicken parts that placed in sterile filtered stomacher bag (Stomacher® 80 Biomaster Bags; Seward Ltd, UK). Treatment was followed accordingly for minced chicken and chicken meat products. Samples were stomached until clumps were dispersed and the whole bag were incubated at 35°C for 20 to 24 hours.

### *Raw beef and beef products, including minced beef*

A total of 75±1.5 mL of modified Tryptone Soy Broth (mTSB; Oxoid, CM0989) were added to approximately 25±2.5 g of raw beef (including minced beef and beef products) placed in sterile filtered stomacher bag (Stomacher® 80 Biomaster Bags; Seward Ltd, UK). Samples were stomached until clumps are dispersed and the whole bags were incubated at 42°C for 15 to 24 hours.

### *Whole chicken carcasses*

A total volume of 400 mL of Buffered Peptone Water (BPW; Oxoid, CM0509B) was poured into the cavity of the chicken carcasses contained in sterile bag (Stomacher® 3500 Series Bags; Seward Ltd, UK). The carcasses were shake for 2 minutes and all the rinsed fluid were transferred to a sterile bag (Stomacher® 400 Bags; Seward Ltd, UK). A total volume of 25±0.6 mL of the rinse fluid obtained was added to 25±0.6 mL sterile BPW and the mixture was incubated at 35°C for 20 to 24 hours.

### *Isolation of Salmonella spp.*

Isolation of *Salmonella* spp. was carried out using immunomagnetic separation (IMS) method. The immunomagnetic beads coated with an anti-*Salmonella* antibody (Dynabead® anti-*Salmonella*, Dynal Biotech ASA, Oslo, Norway) was used in this study and analysis method conducted as per manufacturer instruction.

After immunomagnetic separation process, 50 µL of the IMS beads complex recovered was added to 10 mL Rappaport-Vassiliadis Broth (RV; Oxoid, CM0669), followed by 18-24 hours incubation at 37°C. A loopfull of overnight RV selective enrichment culture were streaked onto three *Salmonella* selective agar; Hektoen Enteric (HE; Oxoid, CM0419), Bismuth Sulphite Agar (BSA; Oxoid, CM0201) and Xylose Lysine Deoxychocolate Agar (XLD; Oxoid, CM0469). All the selective agars were incubated at 37°C for 18 to 24 hours. After incubation, characteristic colonies on the agars were observed.

Typical colonies of *Salmonella* on XLD were pink with or without black centres. Many cultures of *Salmonella* may produce colonies with large, glossy black centres or may appear as almost completely black colonies. On BSA, presumptive *Salmonella* appeared as small black, with metallic sheen while on HE agar it will give greenish blue colonies with a black centre (Ramya *et al.*, 2012; Lee *et al.* 2015). Five suspected colonies (including a typical colony) were selected and inoculate onto Tryptone Soy Agar (TSA; Oxoid, CM0131) for confirmation test and identification of *Salmonella* spp. According to ISO 6579:2002.

### *Confirmation of Salmonella spp.*

Confirmation of *Salmonella* isolates were perform according to International Standard Method (ISO 6579:2002/Amd 1:2007) recommendation. All suspected *Salmonella* colonies were subjected to biochemical test which include Triple Sugar Iron agar (TSI), Lysine Decarboxilase (LIA), urease, indole formation, methyl red and voges-proskauer

reaction, citrate utilization, and simmons citrate. The presumptive positive colonies from biochemical test were subjected to serological test as agglutination with somatic O and flagella H antigens. All observations from confirmation test were recorded and evaluated according to the ISO Standard Method.

### *Identification of Salmonella serovars*

Identification of *Salmonella* serovars were performed using Matrix Assisted Laser Desorption and Ionization Time-Of-Fight mass spectrometry (MALDI-TOF MS; Autoflex, Bruker Daltonic Inc., Germany) and analysed using FlexAnalysis 3.0 software (Bruker Daltonic Inc., Germany). The steps of analysis involved Formic acid extraction of the *Salmonella* isolates and identification of *Salmonella* spp. using MALDI-TOF MS.

### *Formic acid extraction*

Formic acid extraction was performed to extract the biomass in the organic solvent in order to obtain equally distribution of extracted cell for crystallization in the matrix (Böhme *et al.*, 2012). Freshly grown *Salmonella* cultures on Tryptone Soy agar (TSA; Oxoid, CM0131) were prepared for formic acid extraction. The selected colonies were transferred into 1.5 mL micro centrifuge tube and mixed thoroughly in 300 µL of double distilled water. In performing the extraction, the procedure of Formic acid extraction was used as described by Panda *et al.* (2014). The analysis was performed in triplicate as general requirement in quality assurance procedure in laboratory testing.

One microliter (1 µL) of the extracted cell in 70% formic acid-acetonitrile (50:50) solutions was pipetted onto MALDI Target Plate (MTP 384 Target Plate Polished Steel BC, Bruker Daltonic Inc.) and allowed to dry on air at normal room temperature. To prevent any oxidation reaction which can lead to unsuccessfully identifications, 1.0 µL of HCCA (a saturated solution of  $\alpha$ -cyno-4-hydroxycinnamic acid in organic solvent) matrix solution was rapidly overlaid without any delay to the dried supernatant and left to dry at room temperature for several minutes. The plate was then inserted into MTP sample slot on the MALDI-TOF instrument for analysis.

### *Identification using MALDI-TOF MS*

The identification of *Salmonella* species was performed on the MALDI-TOF Autoflex III instrument (Bruker Daltonics, Leipzig, Germany) which equipped with smart beam laser at 200-Hz frequency. FlexControl 3.0 software was used as default setting from manufacturer with ion source 1

Table 1: *Salmonella* spp. detected in retail chicken and beef samples

Sample details	Supermarket		Butcher shop		Wet Market		Total	Total <i>Salmonella</i> detected/25g (%)
	No. of sample	No. of <i>Salmonella</i> detected/25g (%)	No. of sample	No. of <i>Salmonella</i> detected/25g (%)	No. of sample	No. of <i>Salmonella</i> detected/25g (%)		
Raw chicken	24	17 (75.0)	24	12 (54.2)	24	21 (87.5)	72	50 (72.2)
Chicken Minced chicken	12	4 (33.3)	12	5 (41.7)	6	0	30	9 (30.0)
Process products	18	0 (0.0)	18	0 (0.0)	18	3 (16.7)	54	3 (5.6)
<b>Total chicken meat samples</b>	<b>54</b>	<b>24 (44.4)</b>	<b>54</b>	<b>16 (29.6)</b>	<b>48</b>	<b>23 (47.9)</b>	<b>156</b>	<b>62 (40.4)</b>
Raw beef	24	3 (12.5)	24	5 (20.8)	24	6 (25.0)	72	14 (19.4)
Beef Minced beef	12	4 (33.3)	12	1 (8.3)	6	1 (16.7)	30	6 (20.0)
Process products	18	1 (5.6)	18	0 (0.0)	18	3 (16.7)	54	4 (7.4)
<b>Total beef samples</b>	<b>54</b>	<b>8 (14.8)</b>	<b>54</b>	<b>6 (11.1)</b>	<b>48</b>	<b>10 (20.8)</b>	<b>156</b>	<b>24 (15.4)</b>
<b>Total samples purchased</b>	<b>108</b>	<b>29 (26.9)</b>	<b>108</b>	<b>23 (21.3)</b>	<b>96</b>	<b>34 (35.4)</b>	<b>312</b>	<b>86 (27.6)</b>

and 2 at 20kV and 18.6kV respectively. Lens set at 6kV while extractions delay time at 4 nS. 200 laser shots were set for each spectrum with 20 laser shots from different positions of target spot. All intensities results from each shot were collected and analysed.

In identifying *Salmonella* serovars, the peaks list generated were matched against the Biotyper 3.4 database (Bruker Daltonic Inc.) Results of the pattern-matching expressed as score values ranging from 0 to 3. Value of 2.300 to 3.000 indicated highly probable species identification, 2.000 to 2.299 indicated secure genus identification, 1.700 to 1.999 indicated probable genus identification and a score <1.6999 indicated no reliable identification (Cox et al., 2014). Quality control of the spectra was conducted with analysis of the standard mixtures of *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 5462), *Citrobacter freundii* (ATCC11763) and *Salmonella* Typhimurium (ATCC75161).

## Results

### *Occurrence of Salmonella spp. in beef and chicken meat samples*

A total of 312 chicken and beef samples including their process products were examined for the presence of *Salmonella* spp. From total samples analysed, 86 (27.6%) samples found positive for *Salmonella* spp. that were isolated from 24 (15.4%) beef samples and 62 (39.4%) chicken meat samples. *Salmonella* spp. was detected in beef with 14 (19.4%) from raw beef, 6 (20.0%) minced beef and 4 (7.4%) beef products, whereas in chicken meat samples, the presence of *Salmonella* was detected in 50 (72.2%) raw chicken meat samples, 9 (30.0%) minced chicken and 3 (5.6%) chicken meat products. Positive results for *Salmonella* were highest in chicken and beef samples purchased from wet markets (35.4%), followed by

supermarkets (26.9%) and butcher shop (21.3%). Samples obtained from all retail outlets showed highest *Salmonella* contamination in raw chicken meat where percentage of isolation from wet market were 87.5%, 75.0% supermarket and 21.3% butcher shop. Other than raw chicken meat, contamination of *Salmonella* in samples from wet markets were higher in raw beef (25.0%), while samples from butcher shop showed higher detection of *Salmonella* in minced chicken meat (41.7%) and samples from supermarket showed higher detection in both minced chicken meat and minced beef with 33.3%. All results were presented in Table 1.

### *Distributions of Salmonella serovars in chicken and beef*

Identification of *Salmonella* serovars were conducted using MALDI-TOF MS. A total of 90.7 % *Salmonella* isolates gives high probability matching to the library with score value above 2.0, which indicate high probability rate of serovars identification. From 86 *Salmonella* isolated from this study, 8 different *Salmonella* serovars were identified from chicken meat samples and 6 different *Salmonella* serovars were identified from beef samples. Overall, most often *Salmonella* serovars identified were *S. Enteritidis* with 33 (38.4%) followed by *S. Hadar*, 22 (25.6%) and *S. Dublin*, 12 (14.0%). Less than 10% from the total *Salmonella* isolates were identified as *S. Stanley*, *S. Gallinarum*, *S. Anatum*, *S. Choleraesuis* and *S. Typhimurium*. (Table 2).

From a total of 62 *Salmonella* spp. isolated from chicken meat samples, 25 (40.3%) were identified as *S. Enteritidis* followed by *S. Hadar* (24.2%), *S. Dublin* (9.7%), *S. Stanley* (9.7%) and *S. Gallinarum* (9.7%), while *S. Anatum*, *S. Choleraesuis* and *S. Typhimurium* were present and identified in relatively small amount of 2 (3.2%), 1 (1.6%) and 1 (1.6%) respectively in

Table 2: Distribution of *Salmonella* serovars identified from retail chicken and beef samples

Sample (n)*	Sample Type (n)*	No. of <i>Salmonella</i> positive (%)	Identification of <i>Salmonella</i> serovars detected in beef and chicken meat samples							
			S. Enteritidis (%)	S. Hadar (%)	S. Dublin (%)	S. Stanley (%)	S. Gallinarum (%)	S. Anatum (%)	S. Choleraesuis (%)	S. Typhimurium (%)
Chicken (156)	Raw chicken (72)	52 (72.2)	20 (38.5)	14 (26.9)	5 (9.6)	4 (7.7)	6 (11.3)	1 (1.9)	1 (1.9)	1 (1.9)
	Minced chicken (30)	7 (23.3)	4 (57.1)	1 (14.3)	1 (14.3)	1 (14.3)	0	0	0	0
	Process chicken (54)	3 (5.6)	1 (33.3)	0	0	1 (33.3)	0	1 (33.3)	0	0
<b>Total <i>Salmonella</i> spp. detected in chicken samples</b>		<b>62 (39.7)</b>	<b>25 (40.3)</b>	<b>15 (24.2)</b>	<b>6 (9.7)</b>	<b>6 (9.7)</b>	<b>6 (9.7)</b>	<b>2 (3.2)</b>	<b>1 (1.6)</b>	<b>1 (1.6)</b>
Beef (156)	Raw beef (72)	14 (19.4)	4 (28.6)	4 (28.6)	5 (35.7)	0	0	0	1 (7.1)	0
	Minced beef (30)	6 (20)	2 (33.3)	1 (16.7)	1 (16.7)	0	0	1 (16.7)	0	1 (16.7)
	Process beef (54)	4 (7.41)	2 (50)	2 (50)	0	0	0	0	0	0
<b>Total <i>Salmonella</i> spp. detected in beef samples</b>		<b>24 (15.4)</b>	<b>8 (33.3)</b>	<b>7 (29.2)</b>	<b>6 (25.0)</b>	<b>0</b>	<b>0</b>	<b>1 (4.2)</b>	<b>1 (4.2)</b>	<b>1 (4.2)</b>
<b>Total of</b>		<b>Total of</b>	<b>Total of</b>	<b>Total of</b>	<b>Total of</b>	<b>Total of</b>	<b>Total of</b>	<b>Total of</b>	<b>Total of</b>	<b>Total of</b>
<b>Total samples = 312</b>		<b><i>Salmonella</i> detected= 86</b>	<b>33 (38.4)</b>	<b>22 (25.6)</b>	<b>12 (14.0)</b>	<b>6 (7.0)</b>	<b>6 (7.0)</b>	<b>3 (3.5)</b>	<b>2 (2.3)</b>	<b>2 (2.3)</b>

\*n= No. of samples

Table 3: Distribution of *Salmonella* serovars isolated from the samples purchased from different retail outlets

<i>Salmonella</i> serovars distributed in samples, n isolates	Sampling points			Total
	Supermarket (%)	Butcher shop (%)	Wet market (%)	
<b>S. Enteritidis, 33</b>	<b>18 (62.1)</b>	<b>5 (21.7)</b>	<b>10 (29.4)</b>	
Chicken	13	5	7	25
Beef	5	0	3	8
<b>S. Hadar, 22</b>	<b>4 (13.8)</b>	<b>7 (30.4)</b>	<b>11 (32.4)</b>	
Chicken	4	5	6	15
Beef	0	2	5	7
<b>S. Dublin, 12</b>	<b>1 (3.4)</b>	<b>6 (26.1)</b>	<b>5 (14.7)</b>	
Chicken	0	3	3	6
Beef	1	3	2	6
<b>S. Stanley, 6</b>	<b>1 (3.4)</b>	<b>3 (13.0)</b>	<b>2 (5.9)</b>	
Chicken	1	3	2	6
Beef	0	0	0	0
<b>S. Gallinarum, 6</b>	<b>3 (10.3)</b>	<b>1 (4.3)</b>	<b>2 (5.9)</b>	
Chicken	3	1	2	6
Beef	0	0	0	0
<b>S. Anatum, 3</b>	<b>0 (0.0)</b>	<b>1 (4.3)</b>	<b>2 (5.9)</b>	
Chicken	0	0	2	2
Beef	0	1	0	1
<b>S. Typhimurium, 2</b>	<b>1 (3.4)</b>	<b>0 (0.0)</b>	<b>1 (2.9)</b>	
Chicken	0	0	1	1
Beef	1	0	0	1
<b>S. Choleraesuis, 2</b>	<b>1 (3.4)</b>	<b>0 (0.0)</b>	<b>1 (2.9)</b>	
Chicken	0	0	1	1
Beef	1	0	0	1
<b>Total <i>Salmonella</i> isolate</b>	<b>29</b>	<b>23</b>	<b>34</b>	<b>86</b>

n = no of samples or isolates

total *Salmonella* isolates from chicken samples. In beef samples, from a total of 24 *Salmonella* spp. isolated, 8 (33.3%) were identified as *S. Enteritidis* followed by *S. Hadar* (29.2%) and *S. Dublin* (25.0%). Only 1 (4.2%) of the total *Salmonella* spp. isolated from beef identified as *S. Anatum*, *S. Choleraesuis* and *S. Typhimurium*.

From the results, it shows *S. Stanley* and *S. Gallinarum* only isolated from chicken meat samples, where both *Salmonella* serovars were not identified on the isolates from beef samples. *Salmonella* serovars identified associated with process products were *S.*

*Enteritidis*, *S. Hadar*, *S. Stanley* and *S. Anatum*. Other than that, *S. Choleraesuis* was only detected in raw beef and raw chicken, while *S. Dublin* were detected in raw and minced meat samples (Table 2).

#### *Distributions of Salmonella serovars in the samples by type of retailers*

Overall eight *Salmonella* serovars identified in the study were found in chicken meat samples purchased from wet market. However, the presence of *S. Enteritidis* and *S. Gallinarum* were highest in the samples obtained from supermarkets while

Table 4: Distribution of *Salmonella* in packaged and unpacked chicken and beef samples

Sample	<i>n</i> Samples with <i>Salmonella</i> negative (%)	<i>n</i> samples with <i>Salmonella</i> Positives (%)	Total samples
<b>Raw beef, n=72</b>	<b>58 (80.6%)</b>	<b>14 (19.4%)</b>	
Unpacked	13	5 (27.8%)	18
Retailer package	30	6 (16.7%)	36
Commercial package	15	3 (16.7%)	18
<b>Beef (n=156)</b>	<b>24 (80.0%)</b>	<b>6 (20.0%)</b>	
<b>Minced beef, n=30</b>	<b>24 (80.0%)</b>	<b>6 (20.0%)</b>	
Retailer package	7	2 (22.2%)	9
Commercial package	17	4 (19.0%)	21
<b>Process products, n= 54</b>	<b>50 (92.6%)</b>	<b>4(7.4%)</b>	
Retailer package	12	3 (20.0%)	15
Commercial package	38	1 (2.6%)	39
<b>Raw chicken, n=72</b>	<b>22 (30.6%)</b>	<b>50 (69.4%)</b>	
Unpacked	5	28 (84.8%)	33
Retailer package	15	18 (54.5%)	33
Commercial package	3	3 (50.0%)	6
<b>Chicken (n=156)</b>	<b>21 (70.0%)</b>	<b>9 (30.0%)</b>	
<b>Minced chicken, n=30</b>	<b>21 (70.0%)</b>	<b>9 (30.0%)</b>	
Retailer package	5	4 (44.4%)	9
Commercial package	16	5 (23.8%)	21
<b>Process products, n=54</b>	<b>51 (94.4%)</b>	<b>3 (5.6%)</b>	
Retailer package	16	2 (11.1%)	18
Commercial package	35	1 (2.8%)	36

Note: *n* refer as no. of samples

the isolation of *S. Dublin* and *S. Stanley* were highest in the sample from butcher shop. *S. Anatum* was not detected in the samples purchased from the supermarket, while *S. Typhimurium* and *S. Choleraesuis* were not detected in the samples purchased from butcher shops. *Salmonella* serovars detected in chicken and beef samples from wet markets and butcher shops were more consistence compared to supermarket where the highest three serovars isolated were *S. Enteritidis*, *S. Hadar* and *S. Dublin*. For *Salmonella* serovars isolated from supermarket, the highest three serovars identified were *S. Enteritidis*, *S. Hadar*, and *S. Gallinarum*. All the results were presented in Table 3.

#### *Distributions of Salmonella serovars in packed and unpacked chicken and beef*

*Salmonella* spp. detected in packed and unpacked chicken and beef samples from all retail outlets were shown in Table 4. The results shows contamination of *Salmonella* spp. in unpacked chicken and unpacked beef were higher compared to the chicken and beef sold in the packaging. The highest number of *Salmonella* were detected in unpacked chicken meat (84.8%), followed by unpacked beef (27.8%). Chicken and beef sold in retailer's pack gives lower contamination rate compared to the unpacked, while significant reduction of *Salmonella* contamination can be seen in meat purchased with commercial package.

There are only three *Salmonella* serovars identified from unpacked beef; *S. Enteritidis* (6.1%), *S. Hadar* (9.1%) and *S. Dublin* (8.3%), while the

other *Salmonella* serovars identified in unpacked chicken meat includes *S. Enteritidis* (36.4%) *S. Hadar* (27.3%), *S. Dublin* (25.0%), *S. Stanley* (16.7%), *S. Anatum* (33.3%), *S. Gallinarum* (50.0%), *S. Choleraesuis* (50.0%) and *S. Typhimurium* (50.0%). *S. Anatum* and *S. Choleraesuis* were identified in both type of packed beef which is packed by retailer and also commercial packed, while *S. Typhimurium* was identified in commercial packed beef. All identified *Salmonella* serovars were found in packed chicken meat samples except *S. Typhimurium* and *S. Choleraesuis* (Table 5).

#### Discussion

As current practice at the supermarket, frozen beef block were cut into cubes and raw chicken parts were displayed throughout a day, preserved with ice-flake to hold chilled temperature of the meat during sales period. Some beef, minced meat and chicken parts are sold in package which were wrap using cling film, displayed on the refrigerated shelf and normally sold in several days as were stated in the label. Beef and chicken processed products are placed in the freezer, retained as manufacturer packing or in loose items. The frozen condition also applied to the imported beef (with manufacturer packing) while whole chicken carcasses were sold in packaging under refrigerated condition. Proper hygiene condition normally implemented in supermarket with all areas are equipped with air-conditioning.

The condition in the wet market is different, where operation hour for wet market only took 5 to 7

Table 5: Distribution of *Salmonella* serovars in unpacked, retailer package and commercial package chicken and beef samples

<i>Salmonella</i> serovars, n	<i>Salmonella</i> spp. detected in beef			<i>Salmonella</i> spp. detected in chicken meat		
	Unpack (%)	Retailer Pack (%)	Commercial pack (%)	Unpack (%)	Retailer pack (%)	Commercial package (%)
S. Enteritidis, 33	2 (6.1)	3 (9.1)	3 (9.1)	12 (36.4)	9 (27.3)	4 (12.1)
S. Hadar, 22	2 (9.1)	3 (13.6)	3 (13.6)	6 (27.3)	6 (27.3)	3 (13.6)
S. Dublin, 12	1 (8.3)	3 (25.0)	1 (8.3)	3 (25.0)	3 (25.0)	0
S. Stanley, 6	0	0	0	1 (16.7)	4 (66.7)	1 (16.7)
S. Gallinarum, 6	0	0	0	3 (50.0)	3 (50.0)	0
S. Anatum, 3	0	1 (33.3)	0	1 (33.3)	0	1 (33.3)
S. Typhimurium, 2	0	0	1 (50.0)	1 (50.0)	0	0
S. Choleraesuis, 2	0	1 (50.0)	0	1 (50.0)	0	0
<b>Total = 86</b>	<b>5 (5.8)</b>	<b>11(12.8)</b>	<b>8(9.3)</b>	<b>28(32.6)</b>	<b>25(29.1)</b>	<b>9(10.5)</b>

n refers to no. of samples

hours started normally from 7.00 am daily. Chances for microbiological risk were higher as the selling areas normally were uncontrolled in terms of hygiene practice. As described by Vindigni *et al.* (2007) the meat displays area at wet market contained numerous exposure pathways for environmental condition which gives possibility of contamination from rodents and insects. Raw chicken and beef sometimes displayed without ice, exposed on ambient temperature within 27 to 36°C as the outlets normally operate in open space. Only imported frozen beef block, beef and chicken process products were kept in ice box throughout selling period.

In butcher shop normally chicken and beef sold in proper packing and some of the raw and processes meat were produced by the outlet itself. All items were kept frozen throughout selling period. Hygienic condition and handling of the meats at butcher shops were observed better compared to wet market and supermarket. At here, mainly chicken and beef sold were from local farms. The contamination of *Salmonella* in chicken and beef with an overall prevalence of 27.6% indicates the widespread of occurrences and distributions of these pathogens in retails level. A few studies conducted in Vietnam by Van *et al.* (2007) revealed the present of *Salmonella* in retail chicken and beef samples found much higher (53.3% and 62.0%) than reported by Phan *et al.* (2005) which was 21.0% in chicken and 48.6% in beef and 21.0%, supported our findings which the prevalence rate of *Salmonella* in chicken and beef were not significantly different than those reported above with 15.4% and 39.7% respectively. The other report by Vindigni *et al.* (2007) shows 28% of the *Salmonella* were detected from 100 retail chicken meat samples tested in Bangkok, supported contamination of *Salmonella* especially in retail chicken meats were relatively high among the regional countries.

Reported *Salmonella* contamination rate in

retail chicken and beef were significantly higher in most of developed countries. Data compiled by Van *et al.* (2007) and Donado-Godoy *et al.* (2012) reviewed the contamination rate reported in United Kingdom shows 23 to 29% of poultry samples were contaminated with *Salmonella*, 2.8 to 26.4% in Ireland, 13.2% in The Netherlands, 35.8% in Spain, 36.5% in Belgium, 43.3% in Australia, 20% in Argentina, 42% in Brazil, 52.2% in China, 36% in Korea and highest contamination rate reported in Portugal with 60%. In general, climate and storage temperature give an impact to the contamination rates, where tropical country such as Malaysia may lead to replication of *Salmonella* spp. on carcasses faster to the higher average temperature (Van *et al.*, 2007). However, different sampling procedures, sample types (for example whole chicken against chicken part and chilled versus frozen meat), isolation and identification methods could affect the prevalence of *Salmonella* spp. among countries (Dong *et al.*, 2014).

Despite the high percentage of detection in raw chicken and beef that had been expected, the detection of *Salmonella* in minced beef and minced chicken meat also shows a relatively high percentage. Out of 60 minced chicken and minced beef examined, 15 samples (25%) were found to be contaminated with *Salmonella*. The result was significantly lower than the 40% prevalence reported by Ejeta *et al.* (2004). Previous study conducted by other researchers also indicated the occurrence of *Salmonella* spp. in minced meat samples in variable percentage such as 20%, 12%, 12.1%, 11.4%, 6.3%, 6%, 5% and 1.6% (Hassanein *et al.*, 2011), showed the *Salmonella* spp. contamination in minced meat were common as raw meats. Summarized data from several European Countries showed that *Salmonella* prevalence in minced meat range from 0% - 6.8% that were obtained from 280 - 406 minced meat samples collected as per documented in EFSA and ECDC (2012).

*Salmonella* also detected in beef and chicken processed products with prevalence rate of 0.06%. This occurrence describes the correlation between persistence and biofilm formation, which this may be an important factor for increasing tolerance against drying processes and the persistence of bacteria in factory environment (Vestby *et al.*, 2009). Due to the presence of biofilms that protect the bacteria, especially pathogenic bacteria from disinfection and environmental stress, it can support the hypothesis that the formation of biofilms facilitate survival and persistence of bacteria in processed products (Ronner and Wong, 2003; Larsen *et al.*, 2014). In this case, the occurrence of *Salmonella* spp. and increasing numbers of contamination to the processed products by other pathogens might be possible.

Chicken and beef processed products also could be effected with the *Salmonella* contamination during meat processing. After chilling, carcasses normally cut into different parts where meat cutting and de-boning operation involve relatively intensive handling of meat which will increase the microbial risk due to microbial cross contamination via hands and utensils (knives, saws, conveyers etc.) and transfer of bacteria from the chicken and beef surface to the internal parts (Nørrung and Buncic, 2008). Therefore in this case, for meat processed products manufacturing, implementation of HACCP programme is very important to ensure the safety of the processed meat that had been produced.

This finding on prevalence of *Salmonella* in retail outlets were significant with El-Allaoui *et al.* (2013), where the lowest *Salmonella* detection obtained from samples purchased from the butcher shops compared to other outlets. Highest prevalence of *Salmonella* found in chicken and beef sold in wet market might due to unhygienic and uncontrolled environment. The meats were exposed to the open air environment, improper sanitary and the warm temperature with the environment and surrounding were humid (Vindigni *et al.*, 2007). All these factors may promote bacterial contamination and multiplication. The highest prevalence of *Salmonella* in chicken and beef sold in wet market were consistent with the study by Wilson (2002), Zaidi *et al.* (2006) and Vindigni *et al.* (2007) which compared to top supermarkets. Bhattacharya and Dash (2007) were reported the higher rate of *Salmonella* incidence could be attributed to lack of proper cold chains, inadequate power supply and low levels of hygiene in retail outlets.

In wet market, the chicken carcasses were cut into chicken parts using the same cutter and cutting board. Removing the chicken feces from the gizzards using the same utensils contribute to the possibilities

of high contamination of *Salmonella* to the chicken meat. Chicken and beef sold at wet market normally obtained from traditional slaughterhouse with uncontrolled slaughtering and post-slaughtering condition, limited water supply, and regularly used of the recycled rinsing water especially for chicken's carcasses. Meats also being transported to wet market in unhygienic container with inadequate cooling temperature which critical to prevent the growth of microorganism. The multiplication of *Salmonella* in uncontrolled condition during delivery will contribute the spreading of the pathogens among others process products.

High prevalence rate (26.9%) of *Salmonella* detected on the samples from supermarket might due to combination of the low quality of chicken or beef meat from previous day. The cross-contamination might occur from the previous batch or the newer batch of beef or chicken sold on the sampling date. Other than that, cross-contamination also might occur from the other meats or other batches of meats that potentially arose from the equipment or utensils used to prepare meat for sale. Further extensive handling, including slicing into individual part, mincing and packing can lead to cross contamination of meat and meat process products at this retail outlet (Nørrung and Buncic, 2008). Contamination during handling can be due from commercial meat cutter, knife and also unhygienic handling by the workers and contamination by the workers itself. *Salmonella* contamination also could be from the actual infection of food animals at the farm, cross-contamination during slaughtering, distribution and subsequent handling and processing and bring forward to contaminate meat at retail stages (Nørrung and Buncic, 2008). Improper storage with inadequate chilling or freezing temperature would make this worst.

Chicken gizzards that was observed being placed besides of the unpacked chicken parts and beef might contribute to the cross contamination especially at supermarket and wet market. In addition on this, cross contamination of *Salmonella* from gizzard to the chicken carcasses could occur at the early stages from slaughtering house than continued from improper handling, storage, distribution and cutting process at the outlet. El-Allaoui *et al.* (2013) reported that even though chickens meat supplied to the supermarket from established slaughtering house, rupture of the intestine could also occur during evisceration and pooling the giblets might lead to the cross contamination of the chicken carcasses. However, Van *et al.* (2007), was emphasized on the better equipment in slaughterhouses, advanced

processing practices (including the use of dry chilling of carcasses), and more effective use of refrigeration in meat transport as in developed countries could also help to reduce cross contamination of meats. From the study done by Wilfred Ruban *et al.* (2012) indicated the contamination of meat with *Salmonella* was decreased with the increased in sophistication of slaughter facility, where chicken breast and chicken thigh muscles from non-sophisticated outlet shows highest prevalent of *Salmonella* spp. compared to moderate facility outlet, sophisticated outlet and chicken processing plant gives the lowest prevalence.

Pathogen can survive in the food process products, especially in meat until distributed in the markets. Various possibility of contamination and suitable condition in market enhanced the numbers of pathogens to increase and multiply. Dallah *et al.* (2014) mentioned that one of the best method to prevent foods from contamination is the packaging. Therefore, most of food process products in developed and industrialized countries are distributed and sold in proper packing. In Malaysia, most of the manufactured food processed products also distributed and sold in proper packaging as it is required by Food Act 1989 (Act 281) and Regulation. However, some chicken and beef meat process products were sold unpacked in retail stores as it commonly supplied in bulk such as chicken and beef balls and frankfurters. There are also beef and raw chicken process products are prepared and packaged displayed in retail areas such as minced meat and marinated meat. Raw beef and chicken which were displayed and sold unpacked could contribute to the highest possibility of pathogens contaminations. The result of the study by Zhu *et al.* (2014) indicated the prevalence of *Salmonella* contamination among unpacked carcasses (45.1%) was significantly higher than packaged (37.4%) and the observation was aligned with Wang *et al.* (2014) that the packaged was effectively in relative reducing the load and prevalence of *Salmonella* which were attributable to a reduction in the cross contamination during transportation, delivery and retail.

Data from this study showed 22.2% of minced chicken and beef packed by retailers were detected with *Salmonella* compared to 19.0% that commercially packed by established manufactures, and 44.4% of minced chicken meats packed by retailer were at risk compared to 23.8% packed by established manufacturer. In Malaysia, normally processing of minced meat at retailer stage only involved supermarkets and butcher shops. Therefore, this study only presented the data of packed minced meat samples from supermarkets and butcher shops

which given *Salmonella* detection rate 33.3% detected in both minced beef and minced chicken meat from supermarkets, while 8.3% and 41.7% respectively for minced beef and minced chicken meat from butcher shop.

Whole chicken carcasses that sold in the supermarket show less contaminated compared to the carcasses sold in the wet market and butcher shop. This is because the whole chicken carcasses sold at supermarket were supplied by establish company together with original packing, that normally production of fresh chicken meats went through standard treatment to reduce bacteria and pathogen. The treatments involved washing the carcasses using high pressure spray, chilled the carcasses to 4°C within 4 to 8 hours and include sanitizers such as chlorine, acidified sodium chloride, chlorine dioxide, peroxyacetic acid or trisodium phosphate during chilling process (Hugas and Tsigarida, 2008). An application of hygienic approaches and effectiveness of potential interventions during production, slaughtering, manufacturing, preparation and processing of meat process products can significantly reduce the numbers of *Salmonella* positive samples (Van der Fels-Klerx *et al.*, 2008). Persistence of *Salmonella* in food implicated the detection in the imported beef. Although imported beef sold in the proper package, *Salmonella* that already contaminate the beef from imported country could be isolate at retail stage. This were due to the ability of *Salmonella* to survive for prolonged period of time and could readily isolated from samples that had been stored for up to three years (Beuchat *et al.*, 2011).

In this study, *S. Enteritidis* was the most frequently isolated from chicken and beef with isolation rate 38.4% followed by *S. Hadar* (25.6%) and *S. Dublin* (14.0%). *S. Stanley*, *S. Gallinarum*, *S. Anatum*, *S. Choleraesuis* and *S. Typhimurium* were detected in lower rate of 7.0%, 3.5%, 2.3% and 2.3% respectively in both meats. Isolation of various *Salmonella* serotype may pose health hazards especially when the beef and chicken were consumed undercooked and cross contamination might occurs to other foods during meal preparation, storage and handling.

The higher occurrence of *S. Enteritidis* in this study was aligned with Maka *et al.* (2014) and Ramya *et al.* (2012) which reported this serovars as the most predominant in chicken and beef. Finstad *et al.* (2012) also highlighted *S. Enteritidis* was the common with 20% of isolates, while *S. Typhimurium* was the second with 17% isolates where both serovars had involved in most common foodborne outbreak associated with chicken and chicken containing

dishes. However Sallam *et al.* (2014) and Thong *et al.* (2011) were reported high frequency of detection of *S. Typhimurium* which exceeded the detection of *S. Enteritidis* in beef samples.

Result from integrated surveillance of *Salmonella* along the food chain in British Columbia in 2006 until 2010 showed that *Salmonella* had been isolated from 33% of chicken meat and 96% from other meats, with the most observed serovars was *S. Enteritidis* that accounted 39% of total *Salmonella* isolates. Over the year, trend of *Salmonella* isolation and also *S. Enteritidis* were keep on increased, with the report by Gallanis *et al.* (2012), 48% of *S. Enteritidis* isolated from chicken meat found subsequent with 43% on human. This figure gave the perspective that *S. Enteritidis* were spread globally in beef and chicken and may become the major important of *Salmonella* serovars that could contribute to the foodborne illness worldwide.

*Salmonella* Hadar reported by Sarwari *et al.* (2001), was present as the most common trends in human and animal populations. *S. Hadar* was detected in a group of commercial turkeys at United States in the late 1970s and subsequently isolated from food products and poultry. This isolates being the main causes of reported salmonellosis outbreak in humans in 1988. Sarwani *et al.* (2001) gives the relationship between *S. Hadar* and chicken, where contamination of this serovar was decreasing for broilers from 24% in 1990 to 8% in 1995, which may be the reason for the decline of *S. Hadar* isolated from humans in the same period of time. Continuous study of *Salmonella* isolated from food and comparison with human infection would be effective as source of information regarding specific serovar which contributing to Malaysia outbreaks.

Not all *Salmonella* infection is associated with a local products or through the use of local products. In Ireland and United Kingdom, it was estimated ratio of 1: 1 *Salmonella* infection between domestic and imported cases with *S. Enteritidis* and *S. typhimurium* are the main serovar detected in the cases involving imports (Duggan *et al.*, 2012).

Even when food is safe from insidious levels of micro-organisms, poisoning risk still exist. Maintaining standards of hygiene in all aspects of food preparation at home is very important. Ravishankar *et al.* (2010) and Carrasco *et al.* (2012) had reviewed a non-mathematically model of cross-contamination of bacteria from raw chicken to cutting board and from cutting board to vegetables, revealing that from  $10^6$  CFU/g of the bacteria count inoculated on the chicken was transferred to the cutting board and  $10^3$  to  $10^4$  CFU/g from cutting board to the

vegetables. About 40 to 60% of foodborne outbreak cases reported were caused by inadequate handling practices that also includes cross-contamination in between cutting board and cooking utensils (Soares *et al.*, 2012), especially when meat were handled along with other foodstuffs.

## Conclusion

The presence of *Salmonella* in retail chicken and beef including processed products remain a significant public health concerned. This results confirmed retail chicken and beef are the carrier for transmitting foodborne *Salmonella*. Contaminated meats with *Salmonella* at retail point were able to proliferate during storage.

Detection of eight *Salmonella* serovars in this study reflect the possibility of cross-contamination from various sources in slaughterhouses and poor hygiene during the process of cutting meat, contamination during handling and storage as well as retail level. Guidelines for the production and handling of chicken and beef from the farm to the retail stage shall be considered to ensure the safety of meat products were produced for human consumption. The high level of contamination in supermarket and butcher shop require further investigation and sampling the carcasses at established slaughtering houses, could be added to further investigate the *Salmonella* contamination in various level of processing, production and retail.

The isolation of multiple serovars in this study also indicate the risk in public health significantly as chicken and beef are majorly consumed by Malaysian and this could pose the health hazard. Even though in Malaysia chicken and beef are fully cooked before serving for consumption, cross contamination from uncooked meats to ready to eat foods, salads and cooking utensil during meal preparation may also contribute to the risk factors of *Salmonella* contamination and *Salmonella* food poisoning. Such scenarios showed that prevention of cross-contamination in household, personal hygiene, food preparation area and appropriate storage of food should be strictly maintained. These are very important in order to prevent food poisoning salmonellosis occurred with the high prevalence rate of this pathogens on meat, especially raw chicken and beef.

In other words, at every point in food supply chain, possibilities of existence of various types of pathogen contamination should be avoided and awareness of the potential hazard of microorganisms should be enhanced. Precaution should be taken to

guarantee an absolute minimum risk to consumers. Result of this study, along with other findings demonstrated of high prevalence of *Salmonella* in chicken and beef including processed products and also the prevalence of *Salmonella* in the specific retail outlets are suggested a likely linked between human salmonellosis and food of animal origin in Malaysia.

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