

# Bacterial Contamination on Beef Sold at Selected Wet Markets in Selangor and Kuala Lumpur

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## Abstract

Beef is one of the essential sources of protein in human diet. But retail beef are easily contaminated with pathogenic bacteria and can cause foodborne disease. To determine the bacterial contamination, 45 samples of retail beef including imported beef (n = 24) and local beef (n = 21) were collected from selected wet markets at every district in Selangor. Samples were analyzed for total viable counts (TVC), *Escherichia coli*, Enterobacteriaceae and the incidence of pathogenic bacteria which are *Salmonella* spp. and *E. coli* O157:H7. Overall results showed that all beef samples (n = 45) were positive for TVC and Enterobacteriaceae at an average reading of (mean  $\pm$  SD)  $7.05 \pm 0.78$  log CFU/g and  $5.05 \pm 0.87$  log CFU/g, respectively. Only 53.3% of the total samples were contaminated with *E. coli* ( $4.22 \pm 0.60$  log CFU/g) whereas only 24.4% of total samples were found to be positive with *Salmonella* spp. All bacterial count readings fall under the marginal category based on the international standards. There were no significant differences ( $p > 0.05$ ) in microbial counts between the local and imported beef samples for all parameters. Among the *E. coli* isolates detected from the beef samples, 3 isolates were identified as *E. coli* O157:H7. In conclusion, meat safety level for the retail beef sold at wet markets in Selangor and Kuala Lumpur is low and requires more attention from the authorities to ensure its microbiological safety for consumers.

**Keywords:** bacterial contamination, wet market, retail beef

## 1. Introduction

### 1.1 Food-Borne Illness

Food-borne illness caused by the food-borne bacteria had been the crucial global health problems. It could increase the medical cost of individuals and possibly death (Fratamico, 2005). Numerous cases of raw meat as one of the main sources of food borne illness has been reported (Bhandare et al., 2007). *E. coli* O157, *Salmonella* spp., and *Campylobacter* spp., were the most dominant pathogens involved and were often present in fresh meat and poultry (Todd, 1997).

### 1.2 Beef Industry

Beef is one of the major foods of animal origin and has become a part of regular diet for Malaysians. To fulfill the demands of beef products in Malaysia, imported beef supply were imported from other countries such India, Australia, New Zealand, Uruguay, Brazil and Argentina (Ministry of Agriculture and Agro-Based Industry, 2014). This beef products, local and imported are readily sold in wet markets and also supermarkets in Malaysia. However, local beef supply is fairly limited as compared to imported beef as the later are much cheaper and dominates the market (Serin et al., 2008). In Malaysia, most consumers prefer to buy meat from the wet markets because they claimed that all meat at the wet market was fresher than frozen imported meats and was sent directly to the wet markets from the abattoir right after it was being slaughtered (Anjum et al., 2004; Chamhuri & Batt, 2009).

### 1.3 Beef Contamination

Beef available at retail outlets have gone through a long chain process before it is ready at the retailers (Ahmad et al., 2013). The contamination risks were increase during the slaughter and processing of the carcasses (Chapmen 1997). Contaminations also can be compounded during transportation, storage and handling of meat by retailers (Ahmad et al., 2013). This study aimed to assess the contamination of aerobic bacteria, Enterobacteriaceae, *E. coli* and *Salmonella* spp. on local and imported beef from selected wet markets located around Selangor and Kuala Lumpur area.

## 2. Material and Methods

### 2.1 Sample Collection and Preparation

45 samples of beef were collected at a randomly selected wet market in every nine districts of Selangor. Number of beef samples was varied between the wet markets according to the number of vendors available in each wet markets. From the total samples, 21 samples were local beef while 24 samples were imported beef. 100 grams of beef were bought from each beef vendors at the selected wet markets. The samples were then brought to the lab in a cold box and stored at 4 °C. All samples were analyzed in within 48 hrs after collection.

Twenty-five grams of each collected beef samples were weighed and transferred to a sterile bag containing 225 ml of sterilized 0.1% (v/v) buffered peptone water (Merck, Germany). Samples were then homogenized using a stomacher (MiniMix 100PCC Lab Blender, Interscience France) for 2 mins. Ten ml of the homogenized samples were taken out to be used for enrichment steps for detection of *Salmonella* spp. and the remaining samples were used for detection and enumeration of total viable count (TVC), Enterobacteriaceae and *E. coli*. A 10-fold series dilution were performed on the homogenized samples.

### 2.2 Detection and Enumeration of Total Viable Count (TVC), Enterobacteriaceae and *E. coli*

Spread plate technique was used for the enumeration of TVC, Enterobacteriaceae and *E. coli* on Plate Count Agar (PCA) (Merck, Germany), Violet Red Bile Dextrose (VRBD) (Merck, Germany) agar and Eosin Methylene Blue (EMB) (Merck, Germany) agar respectively. A 0.1 ml aliquot from appropriate dilutions was plated on each agar in triplicates. The plated agars were then incubated aerobically at 37 °C for 24 hours. Colony forming unit (CFU/g) were counted and converted to Log<sub>10</sub> CFU/g. Further biochemical tests (Indole, methyl red, Voges-Proskauer and citrate test) were conducted for confirmation of *E. coli* colonies detected on the EMB agar.

### 2.3 Detection of *Salmonella* spp.

Pre-enrichment of the homogenized sample was done by incubating 10 ml of the homogenized sample at 37 °C for 20 hrs. A 0.1 ml aliquot of the pre-enriched samples was then transferred to 10 ml Rappaport-Vassiliadis soy broth (Merck, Germany) and vortex thoroughly before incubated at 41.5 °C for 24 hours for selective enrichment process. After incubation, a loopfull of the enriched broth was then streaked onto Xylose Lysine Deoxycholate (XLD) agar (Merck, Germany) and incubated at 37 °C for 24 hours. Detection of *Salmonella* sp. was recorded by the presence of red colonies with black centers. Further biochemical tests (Indole, methyl red, Voges-Proskauer, citrate and Triple Sugar Iron) were conducted for confirmation of *Salmonella* sp. colonies detected on the XLD agar.

### 2.4 Detection of *E. coli* O157:H7

Confirmed *E. coli* isolates from the biochemical test were further tested to determine the presence of *E. coli* O157:H7. A single colony of the *E. coli* isolates was grown overnight on Tryptic Soy Agar (Merck, Germany) and then streaked onto Cefixime-Tellurite-Sorbitol MacConkey agar in triplicates. The agar was then incubated at 37 °C for 24 hours. Presence of *E. coli* O157:H7 was confirmed with the growth of colourless colonies on the agar.

### 2.5 Statistical Analysis

All results for enumeration of bacterial concentrations were presented as mean  $\pm$  standard deviation (SD). Differences in level of bacterial contamination between local and imported beef were determined using Independent Student's T-test. All statistical tests were performed using SPSS version 22. Results were considered significant at  $p < 0.05$  unless otherwise stated.

## 3. Results

### 3.1 Contamination of Total Viable Counts (TVC), Enterobacteriaceae and *E. coli*

The bacterial loads of total viable counts (TVC), Enterobacteriaceae and *E. coli* of the beef sold at selected wet markets in Selangor and Kuala Lumpur were summarized in Table 1. Overall results showed that all beef

samples ( $n = 45$ ) were positive for TVC and Enterobacteriaceae at an average reading of (mean  $\pm$  SD) log  $7.05 \pm 0.78$  CFU/g and log  $5.05 \pm 0.87$  CFU/g, respectively. Only 53.3% of the total samples were contaminated with *E. coli* (log  $4.22 \pm 0.60$  CFU/g) whereas only 24.4% of total samples were found to be positive with *Salmonella* spp.

Table 1. Microbial load of Total Viable Count, Enterobacteriaceae and *E. coli* on local and imported beef samples sold at wet markets in Selangor and Kuala Lumpur

Beef samples	Microorganisms detected on beef samples					
	Total Viable Count		Enterobacteriaceae		<i>E. coli</i>	
	No. (%) <sup>1</sup>	Mean <sup>2</sup> $\pm$ SD	No. (%)	Mean $\pm$ SD	No. (%)	Mean $\pm$ SD
Local beef	21 (100)	$7.04 \pm 0.78$	21 (100)	$5.03 \pm 0.84$	10 (47.6)	$4.29 \pm 0.53$
Imported beef	24 (100)	$7.06 \pm 0.78$	24 (100)	$5.06 \pm 0.92$	14 (58.3)	$4.17 \pm 0.766$
<b>Total (N = 45)</b>	<b>45 (100)</b>	<b><math>7.05 \pm 0.78</math></b>	<b>45 (100)</b>	<b><math>5.05 \pm 0.87</math></b>	<b>24 (53.3)</b>	<b><math>4.22 \pm 0.60</math></b>

Note. <sup>1</sup> Number and percentage of positive samples; <sup>2</sup> Mean bacterial counts expressed in log CFU/g.

Based on the data (Table 1), all of the bacterial load readings are higher than the limits stated in the International Guidelines for raw beef (Table 2). The average TVC counts for both local ( $7.04 \pm 0.78$  log CFU/g) and imported ( $7.06 \pm 0.78$  log CFU/g) beef have exceeded the acceptable limit according to the Australian guideline (5 log CFU/g). Similar observations were also made on the average reading of Enterobacteriaceae for both local ( $5.03 \pm 0.84$  log CFU/g) and imported beef ( $5.06 \pm 0.92$  log CFU/g) samples which falls under the marginal category based on the EU guideline. The average counts of *E. coli* for both type of samples were also have exceeded the acceptable limit by the Australian Standards (local beef =  $4.29 \pm 0.53$  log CFU/g; imported beef =  $4.17 \pm 0.766$  log CFU/g). From these *E. coli* positive samples, three isolates have been identified as *E. coli* O157:H7.

Table 2. Classification of bacterial counts based on International Standards

Category	Total Viable Count		Enterobacteriaceae		<i>E. coli</i>		<i>Salmonella</i> spp.**	
	AUS <sup>a</sup>	EU <sup>b</sup>	AUS	EU	AUS	EU	AUS	EU
Excellent	< 3.0*	< 2.8	-	< 1.8	Absent	-	Absent	< 2 samples
Good	3.0-4.0	-	-	-	0.0-1.0	-	-	-
Acceptable	-5.0	-	-	-	1.0-2.0	-	-	-
Marginal	> 5.0	> 4.3	-	> 2.8	2.0-3.0	-	Present	> 2 samples

Note. <sup>a</sup> Australian Standards (2002); <sup>b</sup> European Union (2002); - not stated in the guideline; \* unit expressed in log CFU/g; \*\* expressed as presence or absence.

### 3.2 Contamination *Salmonella* spp.

Results for the detection of *Salmonella* spp. were shown in Table 3. About 28.5% of local beef was found to be contaminated by *Salmonella* spp. while 20.8% of imported beef samples were positive with this microorganism. This results also showed that their presence in these samples have violated the standard limits by the International Guidelines. Based on the statistical analysis, this study found that there were no significant differences ( $p > 0.05$ ) in microbial counts between the local and imported beef samples for all microbiological parameters.

Table 3. Frequency of *Salmonella* isolated from local and imported beef samples sold at wet markets in Selangor and Kuala Lumpur

Samples	No. of samples (n)	No. (%) positive with <i>Salmonella</i>
Local beef	21	6 (28.5%)
Imported beef	24	5 (20.8%)
<b>Total</b>	<b>45</b>	<b>11 (24.4%)</b>

#### 4. Discussion

Beef products are easily contaminated with microorganisms due to its nutritious nature and conducive characteristics that favors bacterial growth (Lücke, 2000). Evaluation of the microbiological quality and safety of beef products are commonly carried out by determination of indicator microorganisms' levels such as total viable counts, Enterobacteriaceae and *E. coli* (Moore et al., 2002; EFSA, 2010). The high levels of bacterial counts and also the presence of Enterobacteriaceae in all samples in this study, suggests that there was a high possibility of fecal contamination occurrence on the beef samples and reflects poor general hygiene status of the wet markets. Other than implying that the shelf life of the tested samples was reduced or even unfit to be consumed (Bhandare et al., 2007; Hassan et al., 2010; Ahmad et al., 2013), the high levels of these indicator microorganisms could also indicates the presence of pathogens, for example pathogenic *E. coli* and *Salmonella* spp. (Brown et al., 2000).

To date, there are no microbiological standards specifically for retail raw beef available in Malaysia. Therefore, this study refers to the standards used by Australia and the European Union to interpret the microbiological counts obtained for all parameters. Based on the results obtained, the beef sold at the wet markets in Selangor and Kuala Lumpur falls under the marginal category hence its quality can be deemed as low. Besides poor quality the beef also posed a high risk to the consumers especially if it is not cook properly before consumption. According to the standards, food samples that falls under this category requires corrective actions to be taken such as reviewing the current hygiene level of the beef processing area, training of meat handlers and conduct follow up monitoring to ensure that corrective actions taken are effective (Australian Standards, 2002; EU, 2002).

Presence of *Salmonella* spp. were detected in some of the samples in this study. Australian Standards (2002) has set a zero tolerance policy of *Salmonella* in any retail beef samples as this bacteria is a food-borne disease causing pathogen. Previous study from Stevens et al. (2008), showed that incidence of *Salmonella* sp. on retail beef was higher than on beef samples from the abattoir, suggesting that contamination of this bacteria on retail beef might be mainly due to cross-contamination by the meat handlers at the market or during transportation rather than during slaughter and processing in the abattoir. This study also found presence of *E. coli* O157:H7, an enteric pathogen on the beef samples tested. A study by Radu et al. (1998) which was conducted in Malaysia, had also found *E. coli* O157:H7 in 36% of their tested beef samples. Although there was no *E. coli* O157:H7 outbreak has been reported here in Malaysia, the incidence of food-borne disease caused by this pathogen has been highly reported in countries such as United States and also United Kingdom (Rangel et al., 2005; Locking et al., 2015). Ingestion of *E. coli* O157:H7 is highly dangerous due to its low infectious dose (Desmarchelier & Fegan, 2003) which would lead to hemorrhagic diarrhea or worse, hemolytic uremic syndrome (HUS) and eventually death to the infected person (Karmali et al., 1983).

The initial contamination of beef products usually occurred during the slaughter and processing in the abattoir. However, in this study, there were no significant differences found in the level of bacterial contamination between local and imported beef. Previous study from Stevens et al. (2008), showed that incidence of *Salmonella* spp. on retail beef was higher than on beef samples from the abattoir, suggesting that contamination of this pathogen or other microorganisms on retail beef might be mainly occurred during transportation or due to cross-contamination by the meat handlers at the market rather than during slaughter and processing in the abattoir. This also strengthen the need to maintain a safe food chain as the safety of the beef products can still be compromised along every step of its production.

Various factors could contribute to the increase of bacterial contamination on beef products in wet markets (Sofos, 2008). One of the main contributing factors is temperature where long exposure to unsafe temperature will increase the growth of microorganism on the beef products (Koutsoumanis & Taokis, 2005). According to Rhodes et al. (2009), climate could also contribute to the increase of bacterial contamination in beef as the combination of high temperatures and high humidity throughout the year such as in Malaysia is highly favourable for bacterial growth. The open-air design of the wet markets where appropriate temperature control was not well equipped or even non-existing further increase the risk of temperature abuses as compared to closed building supermarkets or hypermarkets that are more equipped with proper storage facilities with adequate temperature control for their meat. Based on the observation from this study, most beef vendors in the wet markets display their meat products on the open-air retail counter from early morning until closing in the late afternoon, causing the beef products to be exposed to ambient temperature and environment for a long period of time.

Microbial contamination of beef products can also due to the poor sanitary knowledge and practices among the meat handler (Tan et al., 2008). Microorganisms can easily be transferred from the hands of the meat handlers to the beef products. Meat handlers who did not clean their hands thoroughly after visiting the toilet can contaminate their hands with enteric bacteria that then can be transferred to the beef (Taylor et al., 2000; Barza, 2004). Usage of gloves could help in reducing this source of contamination but care must be taken by the meat handlers to change or sanitize their gloves from time to time as the bacteria can accumulate on the glove surface (Lues et al., 2005).

Besides that, contamination could also occur through cross contamination from meat contact surfaces as bacteria may accumulate on the handling tools or equipment and then can be transferred to the beef (Van Asselt et al., 2008). In previous study, cutting and mincing process of retail beef with dirty equipment had shown a significant increase of microbial loads on the minced beef surface as compared to that in whole carcasses (Fenlon et al., 2008). Foodborne pathogens from contaminated meats that transferred to such surfaces can spread infections to the consumers especially if it is not cooked thoroughly (Gorman et al., 2002). The hygiene status of the retail store including the equipment or surfaces used to handle the meat products need to be maintained to prevent continuous contamination of this commodity.

#### 4. Conclusions

In conclusion, this study showed that the quality of beef sold in wet markets around Selangor and Kuala Lumpur are low when compared to the international standard which would increase the safety risk of consumption of this product. Continuous monitoring of this food product as well as the application of hygiene practices along the process from the processing in the abattoir to the retail should be done without fail to ensure the safety of beef supply in Malaysia.

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