Food Research 3 (1) : 28 - 39 (February 2019)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Prevalence and antibiotic profile of Shiga-toxin producing *Escherichia coli* and *Escherichia coli* O157: H7 in beef and buffalo

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Article history:
Received: 28 July 2018
Received in revised form: 26 August 2018
Accepted: 28 August 2018
Available Online: 9 September 2018

Keywords:
Shiga-toxin producing E. coli, E. coli O157: H7, Antibiotic Susceptibility, Polymerase Chain Reaction (PCR), Disk Diffusion Test

DOI:
https://doi.org/10.26656/fr.2017.3(1).218

Abstract

Shiga-toxin producing *Escherichia coli* bacteria are well known to be the pathogenic bacteria that cause traveler diarrhea. *E. coli* O157: H7 from the group of Shiga-toxin producing *E. coli* cause even severe infection which can lead to fatality for humans. In this study, local beef and Indian buffalo were selected to determine the presence of Shiga-toxin producing *E. coli* and *E. coli* O157: H7 using Most Probable Number-Polymerase Chain Reaction (MPN-PCR) method. Among 108 samples, eight (7.41%) samples from local beef and Indian buffalo were detected a positive on *E. coli* O157: H7 while thirteen (12.04%) samples were detected positive for Shiga-toxin producing *E. coli* gene. Out of 108 samples, eleven isolates of *E. coli* O157: H7 were successfully isolated in order to carry out the antibiotic susceptibility test. Shiga-toxin producing *E. coli* isolates were found susceptible to ceftazidime (100%), moxifloxacin (83.33%), sulphamethoxazole (66.67%), ampicillin (50%), amoxycillin (50%), ciprofloxacin (50%), erythromycin (33.33%) and penicillin G (33.33%). *E. coli* O157: H7 isolates were susceptible toward erythromycin (100%), ceftazidime (100%), ciprofloxacin (100%) and moxifloxacin (100%), sulphamethoxazole (60%), ampicillin (20%), amoxycillin (20%), and penicillin G (0%). The safety of both local beef and Indian buffalo was challenged by the presence of both Shiga-toxin producing *E. coli* and *E. coli* O157: H7. Better and safer ways of removing the pathogen from local beef and Indian buffalo should be researched more deeply.

1. Introduction

Shiga-toxin producing *Escherichia coli* (*E. coli*) has been well known as one of the foodborne pathogens which are able to develop numerous outbreaks, especially among children and elders (Gould *et al*., 2009). Large scale outbreaks caused by the Shiga-toxin producing *E. coli* have made this disease visible and created great concern among worldwide. About 5% to 10% of people who were infected with Shiga-toxin producing *E. coli* found developed with the hemolytic-uremic syndrome as well (Thorpe, 2004).

E. coli O157: H7 has been considered as an important indicator bacterium which causes serious and acute disease such as the haemolytic uremic syndrome (HUS), hemorrhagic colitis, and also the thrombotic thrombocytopenic purpura which can lead to fatal in certain cases (Sun *et al.*, 2016). One can be infected with *E. coli* O157: H7 via the consumption of the contaminated food and raw food. The majority of the outbreaks caused by the *E. coli* O157: H7 have been linked to the consumption of the ground beef product (Phillips *et al*., 1996). Cattle are the major reservoir for Shiga-toxin producing *E. coli* O157 (Bono *et al*., 2012). It was found that calves are more easily to be infected with *E. coli* O157: H7 compared to the adult cattle. When one consumed or infected with *E. coli* O157: H7 by consuming the infected beef, intra-family transmission among the family members can happen which lead to a larger outbreak. On the other hand, the contamination of Shiga-toxin producing *E. coli* in the environment is generally from the farmlands which are

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eISSN: 2550-2166

from the feces of contaminated cows, water sources, insects and also wild birds (Vogeleer *et al*., 2014). The mode of transmission of Shiga-toxin producing *E. coli* to human is first via food which is the major vehicle of transmission. Undercooked ground beef is the most common vehicle for transmitting Shiga-toxin producing *E. coli* infection (Jay *et al*., 2004). Other than the transmission via food, direct contact with the infected animal is another mode of transmission. Humans that directly contact with infected farm animals or the contaminated environment will eventually cause Shiga-toxin producing *E. coli* infection on themselves.

Two or more people experiencing the same illness after consuming the same contaminated food or drinks is named a foodborne disease outbreak (CDC, 2011). There are several strains of *E. coli* can cause diarrhea and severe illness to human. *E. coli* is used as a faecal indicator organism (DES, 2003). The presence of *E. coli* in food indicates faecal contamination. *E. coli* should have less than twenty colony-forming unit (CFU) / gram in ready to eat food in order to achieve the satisfactory standard for hygiene (CFS, 2009).

Antibiotics or the antibacterial are the antimicrobial drugs which are used for the treatment or the prevention of infection caused by bacteria. There are several sites of attack for different types of antibiotics. Both penicillin and cephalosporins work by preventing the formation of cell wall, while macrolides, fluoroquinolones and sulphonamides work by preventing the formation of protein, nucleic acid and interruption of metabolism process of bacteria (Pham, 2012).

As time passed, more antibiotics were found and introduced to treat certain infection. Yet, the more the antibiotics, the higher chances of exposure for certain bacteria towards these antibiotics. Vigorous usage of antibiotics results in more resistant bacteria over time. Antibiotic resistance is a situation where bacteria had changed its response towards the use of medicines (WHO, 2016a). From the literature of medical, a drug-resistant bacteria was isolated since the 1970s from a dairy cattle (Sharma, 2017). There were at least 2 million people who were infected with these antibiotics resistant bacteria each year and 23000 people died due to this infection (CDC, 2017b). Infections caused by the antibiotic-resistant bacteria are more difficult to cure. The treatment can be costlier or use a toxic alternative.

Antibiotic resistance *E. coli* was discovered from animals, food and even human. From the study of Meng *et al*. (1998), 118 isolates from *E. coli* O157: H7 and seven isolates from *E. coli* O157: non-motile (designated NM) from animals, food and also human, 24% were resistant to at least one antibiotics. Other than that, 19% were resistant to three or more antibiotics tested. Multidrug-resistant bacteria had now become a serious issue as they have contributed to an increase in morbidity and also mortality (Wood, 2017). Hence, new antibiotics were needed in order to fight infection caused by multidrug-resistant bacteria.

Several prevalence of *E. coli* O157:H7 from beef had been reported in several locations in Malaysia, where 36% detected in retail stores in Malaysia (Son *et al.*, 1998); 9.80% isolated from raw beef in West Malaysia (Apun *et al*., 2006); and 22.6% beef samples imported from Thailand were detected *E. coli* O157: H7 positive (Sukhumungoon *et al*., 2011). However, minimal outbreak of *E. coli* O157: H7 were reported in Malaysia in the past ten years. Hence, this study was carried out to determine if beef supply in Malaysia is hygienic and free from contamination of *E. coli* O157: H7. The aims of this study were to detect and quantify *E. coli*, *E. coli* O157: H7 and Shiga-toxin producing *E. coli* strains in local beef and Indiann buffalo and to determine the antibiotic profile of the Shiga-toxin producing *E. coli* and *E. coli* O157: H7 isolates obtained from local beef and Indian buffalo.

2. Materials and methods

2.1 Sampling and samples preparation

A total of 108 samples were collected and investigated. Among the 108 samples, fifty-six local beef samples and fifty-two Indian buffalo samples were collected randomly over a period of September 2016 to March 2017 from different wet markets in Kajang and Sri Serdang, Selangor, Malaysia. All the samples collected were examined on that day, within an hour, to make sure the freshness of the samples. Samples were randomly chosen from the market in order to give an unbiased result to this study. The sampling process was carried out three times a week in Kajang and Sri Serdang area.

As recommended by Chang *et al*. (2013), 10 g of sample was first added with 90 mL of the Tryptic Soy Broth (TSB) in a sterile stomacher bag. The mixture was then homogenized by using the stomacher for about 1 min.

2.2 Most probable number (MPN)

Three-tube MPN analysis was used, where a 100-fold and also 1000-fold dilutions of the stomached fluid were prepared. For each dilution, one mL was transferred into three sterile MPN tubes containing 9 mL of TSB. All these dilutions were then incubated at 37°C for 24 hrs. Positive tubes from MPN were collected and subjected to DNA extraction and Polymerase Chain

Reaction (PCR) analysis.

2.3 Enumeration of Escherichia coli

Approximately, one mL of the stomached fluid was added with 9 mL of TSB tubes, to obtain10^{-2} dilution. Dilutions were made up to 10^{-9}. An amount of 100 µL of each dilution was pipetted and spread onto Eosin Methylene Blue (EMB) Agar. The plates were incubated in the incubator for 24 hours at 37°C (FDA, 2002).

2.4 DNA extraction

Approximately, one mL was aliquoted from the MPN positive tubes, indicated by turbidity, into 1.5 mL microcentrifuge tubes and centrifuged at 12,000 rpm for 3 mins. The supernatant was discarded and re-suspended with 200 µL of TE buffer. The suspension mixture in the tubes were then boiled at 100°C for 10 mins. The boiled mixture was immediately cooled at -20°C for 10 mins. Lastly, centrifuged at 12,000 rpm for 5 mins and stored in freezer at – 20°C (Chang *et al.*, 2013).

2.5 Multiplex Polymerase Chain Reaction (PCR)

Shiga-toxin genes (*stx*1 and *stx*2), *fliCh7* gene and *rfbO157* gene of stx1-F (5'-ATAAATCGCCATTCGTTGACTAC-3'), stx1-R (5'-AGAACGCCCACTGAGATCATC-3'), stx2-F (5'-GGCACTGTCTGAAACTGCTCC-3') and stx2-R (5'-TCGCCAGTTATCTGACATTCTG-3'), rfbO157-F (5' CGG ACA TCC ATG TGA TAT GG 3'), rfb O157-R (5' TTG CCT ATG TAC AGC TAA TCC 3'), fliCH7-F (5' GCG CTG TCG AGT TCT ATC GAG 3') and fliCH7-R (5' CAA CGG TGA CTT TAT CGC CAT TCC 3') respectively were used in PCR identification (Loo *et al.*, 2013). For multiplex PCR detection of Shiga-toxin genes, a total of 25 µL reaction mixture was used where the mixture contained 5x PCR buffer, 25 mM $MgCl_2$, 10 mM deoxynucleotide triphosphate (dNTP) mix, 1.0 mM of each primer, 1 U of *Taq* polymerase and 2 µL of DNA. While for the detection of *rfbO157* and *fliCh7* genes, a total of 25 µL of reaction mixture was used as well. The mixture contained 5x PCR buffer, 25 mM $MgCl_2$, 10 mM deoxynucleotide triphosphate (dNTP) mix, 1.0 mM of each primer, 1 U of *Taq* polymerase and 2.5 µl of DNA.

The condition used for the amplification of Shiga-toxin gene was started with initial pre-denaturation at 95°C for 5 mins, followed by 30 cycles which including denaturation at 94°C for 30 s, an annealing process at 55°C for 30 s and extension at 72°C for 45 s. Lastly, the process end after the elongation process at 72°C for 7 mins. On the other hand, the condition used for the amplification of *rfbO157* and *fliCh7* genes was the pre-denaturation process at 94 °C for 5 mins. The process was then followed by 35 cycles which including denaturation at 94°C for 1 min, an annealing process at 55°C for 30 s and extension at 72°C for 1 min. Finally, the process end after the elongation process at 72°C for 10 mins.

2.6 Electrophoresis

The PCR products were analyzed via gel electrophoresis using 1.5% agarose gel at 100V. For the product of Shiga-toxin producing *E. coli*, the agarose was electrophoresed for 28 mins, while the product of *E. coli* O157: H7 was electrophoresed for 30 mins. The DNA sizes were visualized under ultra-violet (UV) light in a Gel Documentation System (Syngene, USA).

2.7 Antibiotic susceptibility test

Isolates (n=11) which were successfully obtained from local beef and Indian buffalo were analyzed. Approximately, 50 µL of each isolate from glycerol stock were revived in Tryptic Soy Broth. Isolates were then incubated at 37°C for 24 hrs. Eight antibiotics were used in this study namely erythromycin (E, 15 µg): ampicillin (AMP, 10 µg): penicillin G (P, 10 units); amoxycillin (AML, 10 µg); ceftazidime (CAZ, 30 µg); ciprofloxacin (CIP, 5 µg); sulphamethoxazole/ trimethoprim (SXT, 25 µg); and moxifloxacin (MXF, 5 µg). Disk diffusion method according to the guidelines set by the National Committee for Clinical Laboratory Standard M100 (CLSI, 2016). was used to determine the antibiotic susceptibility prolife. Briefly, by using a sterile swab, the swab was first dipped in the cultures and swabbed evenly on the Mueller Hilton Agar. The agar was then air dried for about a minute. The antibiotics discs were then dispensed onto the surface of the agar. All agar plates were then incubated at 37°C for 24 hrs. *Escherichia coli* ATCC 25922 was used as a control in this susceptibility test. The diameter of inhibition zone of each plate was then recorded. Data collected were then analyzed for the MAR index by using the formula below (Krumperman, 1983):

MAR index = a/b

Where, a = Number of antibiotics where a specific isolate resistant to; b = Total number of antibiotics used in this study.

3. Results

3.1 Prevalence

The presence of bands at 259 bp and 625 bp indicated the presence of O157 gene and H7 gene from *E. coli* O157: H7. Shiga-toxin producing *E. coli* on the other hand produced bands at the 180 bp and 255 bp,

representing the *stx 1* gene and *stx 2* gene respectively. Figures 1 and 2 shows the results of PCR amplified products indicating the presence of *E. coli* O157: H7 and Shiga-toxin producing *E. coli*. ATCC 43895 was used as the positive control.

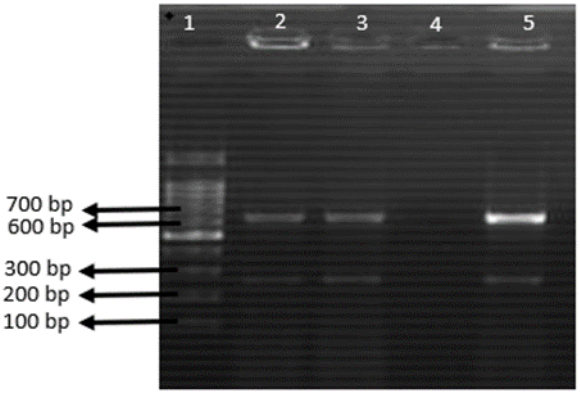

Figure 1. Gel of amplification of PCR-amplified products of *E. coli* O157: H7 at 259 bp (H7 gene) and 625 bp (O157 gene) respectively. L1: Molecular marker 100bp ladder; L2-L3: Positive sample; L4: Negative control; L5: Positive control.

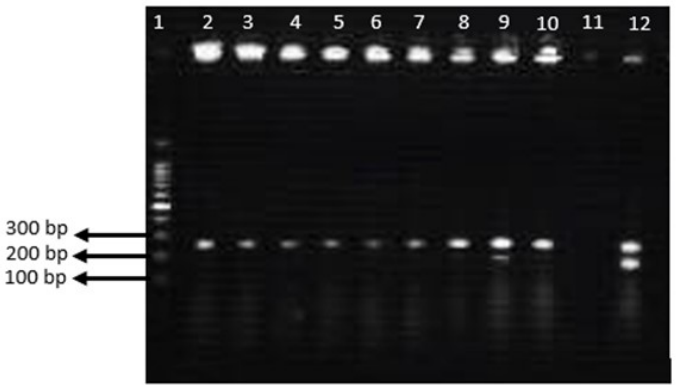

Figure 2. Gel of amplification of PCR-amplified products of Shiga-toxin producing *E. coli* at 180 bp (*stx1* gene) and 255 bp (*stx2* gene) respectively.

Among 108 samples, a total of twenty-one samples were found present with either a single *stx 2* gene or with both *stx 1* and *stx 2* genes. None of the samples were found present with only a single *stx 1* gene. However, most of the samples were found present with only *stx 2* gene, which reached a percentage as high as 85.7% (nine samples from local beef; nine samples from Indian buffalo). The rest of the positive samples were found present with both *stx 1* and *stx 2* gene which was 14.3% (one sample from local beef; two samples from Indian buffalo).

It was found that, eight samples (7.41%) were contaminated with *E. coli* O157: H7, where five samples were from local beef and three samples were from Indian buffalo. On the other hand, Shiga-toxin producing *E. coli* was detected in a higher percentage when compared to *E. coli* O157: H7. There were thirteen samples (12.04%) detected with the presence of Shiga-toxin producing *E. coli*, whereby five samples were from local beef and eight samples were from Indian buffalo.

The microbial load of *E. coli* O157: H7 in the 108 samples fell between the range of < 3 MPN/g to 9.4 MPN/g while the microbial load of Shiga-toxin producing *E. coli* was between < 3 MPN/g to > 1100 MPN/g. The microbial load for both *E. coli* O157: H7 and Shiga-toxin producing *E. coli* in beef and Indian buffalo sample having a minimum and average amount of < 3 MPN/g. The maximum amount of *E. coli* O157: H7 in local beef was 23 MPN/g and 9.4 MPN/g in Indian buffalo. On the other hand, a maximum of >1100 MPN/g of Shiga-toxin producing *E. coli* was identified in both local beef and Indian buffalo samples.

The total plate count for *E. coli* for all samples ranging from 0 colony to as high as 2.63 x 10^8 CFU/g. The highest amount of *E. coli* was found in local beef samples, which reached a value of 2.63 x 10^8 CFU/g while the highest amount detected in Indian buffalo was 7.7 x 10^7 CFU/g. Both local beef and Indian buffalo samples in this case were not much different in their median value which is 3.85 x 10^6 CFU/g for local beef while the latter gave a value of 3.65 x 10^8 CFU/g.

3.2 Antibiotic susceptibility test

A total of eleven isolates were obtained from local

Table 1. Prevalence of resistance to antibiotics for Shiga-toxin producing *E. coli* isolates from local beef and Indian buffalo

Antibiotics	Susceptibility (n (%))		
	S	I	R
Erythromycin (15 µg)	2/6 (33.33%)	0/6 (0%)	4/6 (66.67%)
Ampicillin (10 µg)	3/6 (50%)	0/6 (0%)	3/6 (50%)
Penicillin G (10 units)	2/6 (33.33%)	0/6 (0%)	4/6 (66.67%)
Amoxycillin (10 µg)	3/6 (50%)	0/6 (0%)	3/6 (50%)
Ceftazidime (30 µg)	6/6 (100%)	0/6 (0%)	0/6 (0%)
Ciprofloxacin (5 µg)	3/6 (50%)	3/6 (50%)	0/6 (0%)
Sulphamethoxazole/Trimethoprim (25 µg)	4/6 (66.67%)	0/6 (0%)	2/6 (33.33%)
Moxifloxacin (5 µg)	5/6 (83.33%)	0/6 (0%)	1/6 (16.67%)

S = Susceptible, I = Intermediate, R = Resistant

eISSN: 2550-2166

Table 2. Prevalence of resistance to antibiotics for *E. coli* O157: H7 isolates from local beef and Indian buffalo

Antibiotics	Susceptibility (n(%))		
	S	I	R
Erythromycin (15 µg)	5/5 (100%)	0/5 (0%)	0/5 (0%)
Ampicillin (10 µg)	1/5 (20%)	0/5 (0%)	4/5 (80%)
Penicillin G (10 units)	0/5 (0%)	0/5 (0%)	5/5 (100%)
Amoxycillin (10 µg)	1/5 (20%)	0/5 (0%)	4/5 (80%)
Ceftazidime (30 µg)	5/5 (100%)	0/5 (0%)	0/5 (0%)
Ciprofloxacin (5 µg)	5/5 (100%)	0/5 (0%)	0/5 (0%)
Sulphamethoxazole/Trimethoprim (25 µg)	3/5 (60%)	0/5 (0%)	2/5 (40%)
Moxifloxacin (5 µg)	5/5 (100%)	0/5 (0%)	0/5 (0%)

S = Susceptible, I = Intermediate, R = Resistant

beef and Indian buffalo, where six of them were Shiga-toxin producing *E. coli* positive, while the rest were *E. coli* O157: H7 positive. Tables 1 and 2 show the susceptibility of all isolates toward different antibiotics. Shiga-toxin producing *E. coli* isolates showed highest susceptibility towards ceftazidime (100%), followed by moxifloxacin (83.33%), sulphamethoxazole (66.67%), ampicillin (50%), amoxycillin (50%), ciprofloxacin (50%), erythromycin (33.33%) and penicillin G (33.33%). On the other hand, for *E. coli* O157: H7 isolates showed highest susceptibility towards erythromycin (100%), ceftazidime (100%), ciprofloxacin (100%) and moxifloxacin (100%), followed by sulphamethoxazole (60%), ampicillin (20%), amoxycillin (20%), and penicillin G (0%).

Both erythromycin and penicillin G standard were not found in the Performance Standards for Antimicrobial Susceptibility Testing guideline. However, two Shiga-toxin producing *E. coli* isolates had showed susceptible towards erythromycin, giving a susceptibility range of 20 mm and 12 mm. Two Shiga-toxin producing *E. coli* isolates showed susceptible towards penicillin G, with the susceptibility range of 9 mm and 12 mm. Moving to *E. coli* O157: H7 isolates, all of the isolates showed no resistant towards erythromycin with susceptibility range from 10 mm to 23 mm. However, all of the isolates from *E. coli* O157: H7 were resistant to penicillin G.

Multiple antibiotics resistance (MAR) index and the resistant pattern of each isolates were recorded and analyzed. Most of the isolates showed their resistance towards more than one antibiotics. Since erythromycin and penicillin G were not recorded in the guidelines of Performance Standards for Antimicrobial Susceptibility Testing handbook, the zone of susceptibility appeared on plate will then be considered as resistant towards that specific antibiotic. Table 3 shows the antibiotics resistance pattern and the multiple antibiotics resistance index of Shiga-toxin producing *E. coli* and *E. coli* O157: H7 from local beef and Indian buffalo.

Table 3. Antibiotic resistance pattern and multiple antibiotics resistance index of isolates

Isolates	Resistance Pattern	MAR Index
STEC 61	E15 AMP10 AML10	0.375
STEC 66	E10 P10	0.250
STEC 67	AMP10 AML10 SXT25	0.375
STEC 68	AMP10 AML10	0.250
STEC 75	-	0
STEC 77	P10 SXT25 MXF5	0.375
E 43	E15 AMP10 AML10	0.375
E 62	E15 AMP10 AML10 SXT25	0.500
E 71	E15 AMP10 AML10	0.375
E 73	E15 AMP10 AML10 SXT25	0.500
E 74	E15	0.125

STEC = Shiga-toxin producing *E. coli* isolates; E = *E. coli* O157: H7 isolates

Tested for Erythromycin (E15), Ampicillin (AMP10), Penicillin G (P10), Amoxycillin (AML10), Ceftazidime (CAZ30), Ciprofloxacin (CIP5), Sulphamethoxazole/ Trimethoprim (SXT25), and Moxifloxacin (MXF5).

4. Discussion

Beef is the meat of cattle, where it is categorized under red meat. Beef is a great source where high quality of protein and essential nutrients can be found in it (MLA, 2016). What differentiates between white meat and red meat are their fat contents. White meat is a leaner source of protein, yet with lower fat contents. On the other hand, red meat contains a higher amount of fat, furthermore, they do contain a high amount of vitamins such as the iron, zinc and B vitamins as well (Williams, 2007). In this study, MPN-PCR assay was used. It is simple yet more sensitive compared to conventional plating method (Mantynen *et al*., 1997). MPN-PCR assay can be performed within few hours, while plate counting takes up to two days which is much more time consuming. MPN-PCR method is highly sensitive where it is able to quantify the bacteria population as low as 3 CFU/10 g (Inoue and Nakaho, 2014).

From the result obtained, both Shiga-toxin producing *E. coli* and *E. coli* O157: H7 were found in both local beef and Indian buffalo. In total, thirteen samples (12.04%) and eight samples (7.41%) were detected with the presence of Shiga-toxin producing *E. coli* and *E. coli* O157: H7. Even though both of these pathogens were found in low concentration (<3 MPN/g), yet, a small amount of these organisms are capable of developing the disease in humans where the lowest infectious dose of *E. coli* O157: H7 recorded was 10 to 100 colony-forming-unit (Nguyen and Sperandio, 2012).

Generally, pathogenic *E. coli* is found able to grow at the temperature as low as 7°C to a temperature as high as 46°C, yet with an optimal temperature range of 35°C to 40°C (ICMSF, 1996). However, it was found that *E. coli* O157: H7 was able to survive under the temperature of -20°C for more than 180 days in mangoes and papaya (Strawn and Danyluk, 2010). Shiga-toxin producing *E. coli* is able to survive under harsh condition (Loo *et al*., 2013). Hence, even both local beef and Indian buffalo purchased was store under a temperature of -4°C to maintain its freshness, yet, Shiga-toxin producing *E. coli* and *E. coli* O157: H7 may still able to survive. According to the prevalence study conducted by Cagney *et al*. (2004) 2.70% collected from supermarket, while 3.14% purchased from the butcher shops were positive samples. This showed that, supermarket is practicing a better hygiene practice compared to the butcher shop, which leads to lower prevalence value. In hypermarket, beef and beef products are processed, stored and placed in a cleaner place and ready for sale. This eventually decreased the risk of cross contamination among products. A poor hygiene practice in the wet market will promote the chances of cross contamination. All of the local beef and Indian buffalo were stored in a freezer under -4°C in the wet market before they were sold to the consumers. Poor packaging and lack of hygienic condition of the freezer or even utensils can cause cross contamination. The hygiene of the surrounding and benches in wet market is poor, where flies were found flying around the bench, blood and juices of beef was also accumulated in the basket storing beef. Flies played an important role in transmitting foodborne pathogen. From the study of Jordan and Tomberlin (2017), it was found that, flies gave a higher prevalence of pathogens when compared to other collected samples from kitchen. Other than that, the hands of the worker are a great tool in transmitting pathogenic bacteria as well. A prevalence of *E. coli* O157: H7 on hands of workers in Peninsular Malaysia in abattoirs was done which inferred the possibility. A total of eleven locations had been investigated, there was no *E. coli* O157: H7 isolated from the hands of the abattoirs workers before and after work. Yet, 9.7% out of all of the workers were detected with *E. coli* O157: H7 during works (Shamsul *et al.*, 2016). The absence of pathogen before work indicates the contamination was from the beef itself. This had then become a public health issue where there is a possibility of cross contamination between hand of workers to the other beef or beef products. Workers in wet market were found working with bare hand where no gloves were used during handling or cutting beef. Hence, a poor food handling practice of workers in wet market promotes the transmission of *E. coli* and other pathogens between beef and beef products. Effective cleaning process can reduce the risk of cross contamination and this should be carried out in the end of the day or between every different batches of raw materials to avoid cross contamination. Each utensils and equipment which had either directly or indirectly come in contact with food must be cleaned up properly. Cleaning process should always be followed by sanitizing process to kill bacteria to ensure a clean area to sell and trade food.

In this study, the amount of *E. coli* O157: H7 found was lower in value compared to the amount of Shiga-toxin producing *E. coli* in both local beef and Indian buffalo. From the study of Loo *et al.* (2013), thirty-seven (18.5%) vegetable samples were contaminated with Shiga-toxin producing *E. coli* indicating Shiga-toxin producing *E. coli* showing higher rate of survival. The survival of several types of Shiga-toxin producing *E. coli* which are the *E. coli* O157: H7 and also non-O157: H7 were studied in Argentina (Molina *et al.,* 2003). Several types of Shiga-toxin producing *E. coli* non-O157 strains were used which were the *E. coli* O26: H11, O88: H21, O111: H¯, O113: H21, O116: H21, O117: H7, O171: H2 and OX3: H21. pH test was conducted, and the pH reported was 2.5 for 8 hrs to test out the acid tolerance of all of the strains. However, none of the bacteria survived, except the *E. coli* O91: H21 where it survived longer than 24 hrs. Other than that, ethanol test was carried out at the same time as well to determine the alcoholic resistance of all these strains. There was no survival found after 24 hrs incubation in Luria Bertani broth which had been supplemented with 12% of ethanol. Yet, all the strains were found resistant to 6% ethanol. Among all strains, *E. coli* O91: H21 showed the highest resistance. Hence, Shiga-toxin producing *E. coli* (*E.* coli non-O157) showed a greater survival rate in harsh environment compared to *E. coli* O157: H7 which gave a good explanation on the higher amount of Shiga-toxin producing *E. coli* found in both local beef and Indian buffalo in this study.

A total of twenty-one strains were found carrying *stx1* and *stx2* gene. Among them, eight were classified as O157: H7, while the other thirteen were revealed as the non-O157: H7. Both *stx1* and *stx2* genes were detected

in three out of these twenty-one strains, while the others were detected to be present with only stx2 gene. From the study of Tahamtan *et al*. (2010), a similar result was reported, where only the *stx*2 gene were present. Some studies had shown that strains possessing only *stx2* gene are more virulent compared to strains carrying *stx1* gene or both *stx1* and *stx2* genes (Nataro and Kaper, 1998; Ludwig *et al*., 2002). *Stx2* gene was found to be 400 times more toxigenic than *stx1* gene (Riley *et al.*, 1982).

The *E. coli* contaminated local beef and Indian buffalo may indicate fecal contamination. Local beef has slightly higher number of *E. coli* compared to Indian buffalo. *E. coli* contamination might occur at different phase. They can be contaminated either from the infected organisms or the environment. Cross contamination is the main risk factor of transmitting *E. coli* to the other in the slaughter or even during the processing and storing stage. This can happen either in the slaughter house or in a retail shop, wet market or hypermarket due to low hygiene practice (Heinz, 2008). Other than that, beef and buffalo can get infected with *E. coli* via consuming contaminated irrigated water or from the surroundings (Fairbrother and Nadeau, 2006). These bacteria may colonize the gastrointestinal tract of the cattle and buffalo, and then contaminate the carcass of the beef or buffalo during slaughter or processing (Reinstein *et al*., 2007).

Antibiotic treatment for *E. coli* O157: H7 infection is still questionable as treating *E. coli* O157: H7 is possible a factor causing HUS (Smith *et al*., 2012). However, this phenomenon appeared to be linked to certain antibiotics and specific pathogens (Mor and Ashkenazi, 2014). Antibiotic treatment used for Shiga-toxin producing *E. coli* requires more research. According to German researchers, azithromycin was able to ease severe *E. coli* infections (Steven, 2018) where patients treated with this antibiotic were found free from pathogenic bacteria after full treatment. A total of eight antibiotics were chosen for this study. Antibiotics used in this study were selected from different classes such as macrolides, penicillin, cephalosporins, fluoroquinolones and sulfonamides. Each class has different counter effects either inhibiting the synthesis of cell wall, protein, nuclei acid, or interrupting the metabolism of the bacteria.

E. coli is a Gram-negative bacterium and hence, erythromycin and penicillin G will have no effect on *E. coli*. The susceptibility range for these antibiotics were not described in the Performance Standards for Antimicrobial Susceptibility Testing guideline. Gram-negative bacteria have an additional protection of the outer membrane which is a bilayer of glycolipid lipopolysaccharides and glycerol phospholipids (Miller, 2016). This membrane acts as a strong barrier towards toxic compounds, causing bacteria to be resistant to more antibiotics. Beta lactam antibiotic is a class of antibiotics where they contain a beta-lactam ring in their molecular structure. Penicillin is one of the derivatives that is grouped under this class of antibiotic. However, *Enterobacteriaceae* is able to produce a type of enzyme named the extended-spectrum β-lactamases (ESBL), which can cause these bacteria to be resistant towards beta lactam antibiotics by hydrolyzing the beta lactam ring in the antibiotics (Shaikh *et al*., 2015).

From the result, 33.33% of Shiga-toxin producing *E. coli* were found susceptible towards penicillin G which is a type of antibiotics under penicillin. In the year of 2009, penicillin had become obsolete as they had no longer effective in common disease due to their resistant towards penicillin (Tutton, 2009). Over the time, some strains under *E. coli* have begun having susceptibility toward penicillin. However, none of any isolates from *E. coli* O157: H7 from this study was susceptible to penicillin G. All the isolates from *E. coli* O157: H7 remain highly resistant toward penicillin G. The result was in the agreement with Mustika *et al.* (2015), where all of the *E. coli* O157: H7 isolates were fully resistant toward penicillin G. Other than that, ampicillin and amoxycillin are under the same group as penicillin whereby the mechanism of their action is by inhibiting the synthesis of cell wall. As discussed before, as *E. coli* is a Gram-negative bacterium, they have a strong outer membrane which enables them to resist the effect of antibiotics. Hence, half of the isolates from Shiga-toxin producing *E. coli* were resistant to both antibiotics. On the other hand, 80% of the isolates from *E. coli* O157: H7 were resistant to both antibiotics. Gram-negative bacteria will show high resistant towards antibiotics which inhibit the synthesis of cell wall. Resistance of Shiga-toxin producing *E. coli* remains high from the result of Rao *et al*. (2011), where half of the isolates were resistant to both ampicillin and amoxycillin. Even though there were result showing Shiga-toxin producing *E. coli* had finally shown some susceptibility to antibiotics under penicillin group, yet, these bacteria still showed high resistant to these antibiotics. Antibiotics which attack bacteria from cell wall will be less efficient.

Moving to antibiotics under group of macrolides, which inhibit the synthesis of protein within the bacteria by binding itself to the 50S subunit of the bacteria ribosomes so that binding of tRNA is blocked (DrugBank, 2017). Erythromycin is known to have no effect on *E. coli*, where *E. coli* have high resistant toward this antibiotic. This can be seen since the finding from Andreont *et al*. (1986), where *E. coli* was found resistant to erythromycin, even in high levels. This was due to the

synthesis of erythromycin esterase which was able to inactivate the antibiotic hence giving no effect to the bacteria. Out of the six isolates of Shiga-toxin producing *E. coli*, four isolates were resistant to erythromycin. The study became interesting when all the isolates of *E. coli* O157: H7 were found susceptible to erythromycin, where they showed an inhibition range from 10 mm to 23 mm on the agar plates. The same finding was discovered by Tanzifi *et al.* (2015) where only 30% of *E.* coli from raw milk were resistant towards erythromycin. A lot of study had shown that *E. coli* O157: H7 was totally or strongly resistant to erythromycin (Reuben and Owuna, 2013; Dulo *et al*., 2015; Enem *et al*., 2017), with minimal research showing *E. coli* was susceptible to erythromycin. Various reasons might lead to the susceptibility of *E. coli* O157: H7 to erythromycin. Microbes are dynamic living things, where they might change in term of characteristic, genetic and other from time to time. For the past few years, *E. coli* had reported to be resistant to erythromycin, yet, from this research, the results were totally different.

Cephalosporin is a class of beta-lactam antibiotics which is similar to penicillin. It does have the same mode of action as the other beta-lactam antibiotics, however, they are more resistant to beta-lactamases produced by the bacteria which causes the antibiotic to be ineffective. Cephalosporin have a higher resistance towards beta-lactamase and hence have greater effect in inhibiting the cell wall synthesis of bacteria (Goodwin and Hill, 1976). In this study, ceftazidime was used to study the susceptibility of isolates. From the results, all the isolates from Shiga-toxin producing *E. coli* and *E. coli* O157: H7 were 100% susceptible to this antibiotic, which indicates beta-lactamases resistance antibiotics are more effective in treating the infection from Gram-negative bacteria such as the *E. coli*.

Quinolones are one of the very effective classes of antibiotics for the treatment of infection is human. Fluoroquinolones are the introduction of fluorine to the skeleton of quinolones (Aldred *et al*., 2014). Quinolones work by binding themselves to the enzyme which is the DNA gyrase or the DNA topoisomerase that is responsible for the unwinding process of DNA during replication, causing DNA unable to replicate (Mehta, 2011). Ciprofloxacin and moxifloxacin are the antibiotics used in this study which grouped under quinolones where both work by inhibiting the synthesis of DNA in bacteria. Both antibiotics showed great results in this study, where 0% of isolates from both Shiga-toxin producing *E. coli* and *E. coli* O157: H7 were resistant to ciprofloxacin. On the other hand, there was only 16.67% of isolates from Shiga-toxin producing *E. coli* resistant to moxifloxacin and none of any isolates from *E. coli* O157: H7 were resistant to moxifloxacin.

Finally, sulfonamides work by inhibiting metabolic process in bacteria. In this study, trimethoprim was the antibiotics used under this group. There were 2 isolates from both Shiga-toxin producing *E. coli* (33.33%) and *E. coli* O157: H7 (40%) were resistant to this antibiotic where this antibiotic is still considered effective towards gram negative Shiga-toxin producing *E. coli* and *E. coli* O157: H7. There are various ways where antibiotic resistance can spread among livings. Overuse or misuse of antibiotics on animal or human will cause the development of antibiotic-resistant bacteria in the guts of the living. Feces from such animals can carry these antibiotic-resistant bacteria as well. Fertilizer or contaminated water absorbed by the crops or eaten by animal or human can then obtain these bacteria. Human who are infected can spread these bacteria when they are in nursing home or hospital. These bacteria spread to the other patients or staff from surface of equipment in hospital (CDC, 2017a).

The MAR index for all isolates were analyzed as well. The results obtained showed a range of MAR index from 0 to 0.50. Most of the MAR index for Shiga-toxin producing *E. coli* were higher than 0.2. In parallel, almost all of the isolates from *E. coli* O157: H7 were having a MAR index higher than 0.2. Overall, 81.82% of all isolates were having a MAR index greater than 0.2 which were suggesting that their origin were from a high risk source of contamination (Paul *et al*., 1997).

5. Conclusion

In conclusion, both Shiga-toxin producing *E. coli* and *E. coli* O157: H7 were detected in both local beef and Indian buffalo marketed in wet market in Selangor area. This finding indicated the presence of risk for *E. coli* infection in Malaysian even the least infection case was reported. *E. coli* count in both local beef and Indian buffalo is considered high, showing poor hygiene of meat in Selangor area which eventually will increase the risk of cross contamination and *E. coli* infections among consumers. Several strains collected from beef and Indian buffalo surprisingly showed susceptibility toward penicillin which had been well known to be ineffective towards Gram-negative *E. coli*. This phenomenon indicates more study on antibiotics treatment is needed for *E. coli* infection as bacteria are dynamic. Antibiotics from cephalosporin had shown the best result towards Shiga-toxin producing *E. coli* and *E. coli* O157: H7 strains. Antibiotics treatment towards *E. coli* infection can be very subjective. Further study should be carried out in order to identify the best antibiotics treatment for *E. coli* infections. Minimal cases reported does not mean

no outbreak will occur. Good hygiene practice should be introduced to food processors in order to reduce outbreak, at the same time, authorities involved should be putting more effort in providing more campaigns or seminars in order to increase public awareness on food safety and the proper way in handling food to ensure a safe farm to fork food.

Acknowledgement

This research was financially supported by Research University Grant Scheme Initiative Six (RUGS 6) of Universiti Putra Malaysia (GP-IPS 9438703 and GP-IPS 9466100) and Fundamental Research Grant Scheme (FRGS) of Ministry of Higher Education (MOHE), Malaysia (02-01-14-1475FR).

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