Evaluation of Bacterial Contamination on Local and Imported Mutton in Meat Markets in Benghazi - Libya

ABDALLA MOHAMMED ABDALLA MANSOUR[1]*, ADEL M. MILAD ISHLAK[2], BAKKAR ALI HAJ-SAEED[2]
[1]Department of Food Industry, Faculty of Agriculture, University of Benghazi, Benghazi, LIBYA
[2]Department Animal Production, Faculty of Agriculture, University of Benghazi, Benghazi, LIBYA
Benghazi - Libya
*Email: Abdalla.mansour@uob.edu.ly

Abstract: - The study was conducted to evaluate the bacteriological contamination (total bacteria counts (TBC), total coliform counts (TCC), total staphylococcus counts (TSC) and Salmonella) of mutton in meat shops in Benghazi city, northeast of Libya, where the city was divided into five sectors (A, B, C, D, and E). The investigation in the sector (A) showed that the highest average of TBC in local and imported meat was (7.99 and 7.19 $\log_{10}$CFU/g) respectively, which was a statistically non-significant difference ($P \leq 0.05$). The experiment reported that the lowest average of TCB in local meat was a non-significant difference ($P \leq 0.05$), which was in the sectors (C&D) (5.78 and 5.64 $\log_{10}$CFU/g) respectively. Coliform bacteria, the results showed a significant difference ($P \leq 0.05$) between local and imported sheep meat in all sectors (7.15, 6.78, 6.26, 6.48, 5.11, 4.46, 4.41, 4.43, 6.01, 6.30 $\log_{10}$CFU/g) respectively. On the other hand, *Staphylococcus aureus* was a significant difference ($P \leq 0.05$) between local and imported sheep meat in all sectors. Whereas, the sectors (A) local meat and (B) imported meat exhibited that the highest average of TSC was (6.87 and 6.97) respectively. The biochemical test showed numbers of bacterial species: *E. coli, Enterobacter spp., Citrobacter spp., Klebsiella spp., Proteus spp., Pseudomonas spp., Salmonella spp*. Salmonella bacteria have appeared in 5.7% of isolates in Sector B. Accordance with the FAO and Libyan standards, the study reported that 40% of the studied samples were below the microbial limit, while 60% of the samples exceeded the maximum limit. From the results, the researcher concludes that there is a weakness in the application of sanitary procedures in meat shops and slaughterhouses in the city of Benghazi.

Key-Words: - Mutton – Coliform – *E. coli* - *staphylococcus aureus* – *Salmonella* – contamination – Benghazi.

1 Introduction

Traditionally in Libya, people prefer to eat lamb meat instead of beef meat. Libyans use lamb meat in social events. Hence, the meat is considered the most valuable animal product. Nutritionally, meat consists of protein, amino acids, mineral salts, fats, fatty acids, vitamins and other active biological components, as well as small amounts of carbohydrates [1]. The importance of meat in nutritional terms stems from the high-quality protein available in it for containing all essential amino acids, as well as the mineral salts and bio-vitamins readily available [1]. FAO report in 2014 showed that the average per capita consumption of meat in the developed world was 76 kg/year and that in the developing world it was 33 kg/year [2].

The meat is a source of contamination from slaughtering until the end of selling [3]. The bacterial load at the surface of sheep carcasses is essential to evaluate that to deal with the international standards [4, 5]. The existence of various bacteria on meat is an indication of low standard levels of animal's hygiene and the handling of meat from pre-slaughter to post-slaughter, abattoir facilities and sales of meat [6]. In the same content, microorganisms present in the meat may be harmful to human, and cause spoilage and may be used as an indicator. Various types of bacteria were isolated and identified on fresh meat [4].

There are four major pathogens that have frequently been associated with meat and meat products including *Salmonella spp., Campylobacter spp., Listeria monocytogenes, and Escherichia coli O157:H7* [7].These organisms have been linked to a number of cases of human illness [8]. The Enterobacteriaceae family contains a large number of organisms, some of the non-fecal origin, that are useful as an indicator of the overall process hygiene in the abattoir. *E. coli* is the indicator of bacteria of choice associated with feces [9, 10, and 11]. Several researchers have reported that the meat samples were contaminated with high level *of Klebsiella*

pneumoniae, Enterobacter spp., Pseudomonas aeruginosa, E. coli, Salmonella spp., Serratia marcescens and Proteus vulgaris, Staphylococcus aureus and Bacillus spp. [12, 13]

In Libya, a study showed that cooked meat samples were contaminated with *E. coli, E. coli 0157:H7, Staphylococcus aureus, Aeromonas spp., and salmonella*, while uncooked samples were extremely contaminated with *Escherichia coli Aeromonas spp., Staphylococcus aureus, E. coli 0157:H7 and salmonella* [14]. Researcher reported that Salmonella species had the highest value of 15 (50.1%) from the market, found in sheep, while the lowest occurrence of Salmonella species was associated with 3 (10.0%) in goats sampled from shop meat [15]. Experiment observed 44% *Enterococcus faecalis, 40% Klebsiella oxytoca, E. coli 10%, Pseudomonas mirabilis 4%* and some unidentified bacteria in meat samples [16].

The Objective of the study is to knowledge types of the pathogenic bacteria associated with these meats as Coliform, Salmonella and Staphylococcus aureus, then a comparison of the upper limit of these bacteria with the upper limits of Libyan and FAO specifications. Through the aforementioned objectives, the researcher can be to know the extent to which health measures are applied in meat shops and slaughterhouses.

2 Materials and Methods

2.1 Samples Collected

Meat samples were collected from meat markets in Benghazi, in the northeast of Libya, where the city was divided into five sectors (A, B, C, D, and E). From each sector, samples of local mutton and imported mutton were taken. The fresh mutton samples (domestic & imported) were immediately sent refrigerated in an icebox within one hour of collection to the microbiology laboratory at Agriculture College - Benghazi University.

2.2 Processing of Samples for Analysis

Upon arrival at the laboratory, each sample was assigned an individual unit number and was analyzed as a discrete unit as follows:

1- 25 grams of each meat sample was aseptically transferred to a sterile blending container, previously sterilized by washing with hot water and rising with 95% ethanol alcohol and then allowing the remaining alcohol to burn. The sample was blended with 225 ml of sterile nutrient broth for 2 minutes to obtain a homogenate mixture with 0.1 ml (1/10) dilution [17].

2- The homogenate mixture was aseptically transferred to a sterile 500 ml bottle having the sample number, mixed well by swirling the bottle. Then the bottle cap was loosening, and incubated at 37°C for 24 hr., for the isolation of salmonella [18].

2.2.1 Plate Count Agar (PCA)

Includes the following steps:

A. 1ml of homogeneous mixture sample (0.1) was transferred aseptically to a tube containing 9 ml of nutrient broth to obtain a concentration of 0.01 (1/100).

B. 1 ml aliquots from diluted tube to a blank nutrient broth tubes using new tips each time to get the dilutions to 10^{-6}

C. 1 ml of each dilution was transferred into three sterilized petri dish, About 10 to 15 ml of Plate count agar medium tempered to 45°C was poured into plates, and the contents of the plates were mixed thoroughly with a diluted sample.

D. The dishes were incubated for 24 - 48 h at 37°C [19].

E. After incubation, the number of colonies (CFU/g) was counted, using a plate with 25 - 250 colonies [20].

To prove the total number of bacteria, the number of poured plates containing 30 to 300 colonies was used to estimate the total number of bacteria in meat samples. The total bacteria counts were calculated by multiplying the inverse dilution factor by the average number of colonies in the plates (three replicates per dilution). Colony formation unit /gram (CFU/g) of the sample was converted into $\log_{10}$CFU/g.

2.2.2 Total Coliform Count Test (TCC)

TCC was tested using the method described by [18].

I. 1 ml of each dilution was transferred into three sterilized petri dish

II. About 10 to 15 ml of violet red bile (VRB) agar, tempered to 45 °C, were poured into plates, the contents of the plates were mixed thoroughly by using conventional mixing procedures, and allow solidifying (5 to 10 minutes) on a level surface.

III. Duplicate plates and agar control plates were run for each series of samples.

IV. The number of samples was selected to be plated in any one series, so there was no more than a 20 minutes time lapse between diluting the first sample and pouring the last plate in the series.

V. The plates were then inverted and incubated for 24 hours at 35°C.

VI. Coliforms in VRBA appear as typical dark red colonies normally measuring at least 0.5 mm in diameter on uncrowded plate [21].
VII. After incubation, the number of coliform colonies (CUF/g) was counted, using plates with 15 - 150 coliform colonies [21].

2.2.3 Identification of Coliform Bacteria

Four well-separated dark red colonies were suspected to be coliform from uncrowded violet red bile agar. Typical colonies were confirmed by staining followed by different biochemical tests. All the media were obtained from Oxoid limited, England. The different isolates colonies were confirmed by staining, microscopic, cultural and biochemical tests (motility test, catalase test, oxidase test, citrate utilization, indole test, MRVP test, and triple sugar iron (TSI) test). [20, 21, 22 and 23].

2.2.4 Isolation and Identification of Staphylococcus Aureus

1. Using the appropriate pipette, 0.1 ml of both dilutions 10^{-3} and 10^{-4} transferred to the surface of prepared plates from the Staphylococcus medium No-110 (gelatin mannitol salt agar) media for the development and selection of Staphylococcus bacteria - two replicates per dilution.
2. The amount transferred from the dilution distributed by L-shaped glass rod on the surface of the hardened media, then incubated at 35°C for 24 hours. The resulting yellow color colonies appear on media of Staphylococcus bacteria, which refer to the presence of Staphylococcus aureus [24, 25].

2.2.5 Isolation and Identification of Salmonella

Promote bacterial growth:
1) The remaining of 1/10 dilution of the sample is taken with the nourishing broth, placed in a sterile flask and incubated at 37°C for 24 hours.
2) After incubation, the preparation is mixed and is taken a full needle and aseptically struck on S.S. Agar and Xylose Lysine Deoxycholate (XLD) Agar and incubate dishes at 37°C for 24 hours.
3) At the end of the incubation period, the dishes are tested for the presence of Salmonella, where their colonies appear on the XLD media in the form of pink-red colonies with or without a black center, while the S.S Agar environment appears colorless in a black center or without it
4) Typical colonies were confirmed by staining followed by different biochemical tests.

3 Data Analysis

The data were analyzed with SPSS software (Statistical package for social science version 23, IBM/SPSS). Descriptive statistics were used to analyze the data. In addition, all bacterial counts were converted to $\log_{10}$CFU/g for analysis. ANOVA was performed. Duncan's test was used as a post hoc test. Mean differences were considered significant at $p < 0.05$. The comparison was between rows and columns.

4 Results

The results of this study showed the following:

4.1 Total Bacteria Counts (TBC)

Results of TBC, statistical tests showed that there were significant differences ($P<0.05$) between all sectors as well as between local and imported meat. Tables 1 show the average total number of bacteria in meat samples studied in the five sectors. Examine local meat showed that the mean the total number of bacteria in all sites was (7.99, 7.4, 5.78, 5.64, and 7.73 $\log_{10}$CFU/g) respectively. The highest mean number of TBC in local meat was 7.99 in the site (A), while the lowest number of TBC was (5.64 $\log_{10}$CFU/g) in the sector (D).
Bacterial tests of imported sheep meat showed significant differences ($P<0.05$) between all sectors. Imported mutton meat, the average of TBC were (7.91, 7.22, 5.96, 6.05, and 6.76 log10CFU/g) respectively. The highest and lowest average TBC was in the sector (A) and (C).

4.2 Total Coliform Counts (TCC)

Table 2 (TCC), vital statistical analyses exhibited that there were significant differences ($P<0.05$) between all sectors in addition to between local and imported meat. Examination of local meat (Table 2) shows that the average of TCC in each sector was (7.15, 6.26, 5.11, 4.41, and 6.01 $\log_{10}$CFU/g) respectively. Analysis of imported lamb meat (table 2), the results were fairly close to the results of local mutton and were as follows as (6.78, 6.48, 4.46, 4.34, and 6.30log10CFU/g) respectively. The largest and smallest mean of TCC in local and imported meat was in the sector (A) and (D) (7.15 and 4.41 $\log_{10}$CFU/g) (6.78 and 4.34 $\log_{10}$CFU/g) respectively

Table 1: Average total TBC (log_{10}CFU/g) of mutton meat in Benghazi markets

Sectors	Local mutton	Imported mutton
A	7.99 a	7.91 a
B	7.41 c	7.22 d
C	5.78 h	5.96 g
D	5.64 h	6.05 g
E	7.73 b	6.76 f
FAO standard	7.00 e	7.00 e

Mean counts with the different letters are significantly different at p≤0.05.
The comparison was between rows and columns.

Table 2: Average of TCC (log_{10}CFU/g) of mutton meat in Benghazi markets

Sectors	Local mutton	Imported mutton
A	7.15 a	6.78 b
B	6.26 cd	6.48 c
C	5.11 e	4.46 f
D	4.41 f	4.34 f
E	6.01 d	6.30 c
FAO standard	0.00 g	0.00 g

Mean counts with the different letters are significantly different at p≤0.05.
The comparison was between rows and columns.

Table 3: Average of TSC (log_{10}CFU/g) of mutton meat in Benghazi markets

Sectors	Local mutton	Important mutton
A	6.87 ab	5.00 e
B	6.57 b	6.97 a
C	5.60 c	5.40 d
D	5.77 cd	5.60 cd
E	5.57 cd	5.67 cd
FAO standard	0.00 f	0.00 f

Mean counts with the different letters are significantly different at p≤0.05.
The comparison was between rows and columns.

4.3 Total Staphylococcus Aureus Counts (TSC)

The TSC tests (table 3) showed that there are significant differences (P < 0.05) between all sectors and between meat types. In each sector, the averages of TSC (local meat) was (6.87, 6.57, 5.60, 5.77, and 5.57 log_{10}CFU/g) respectively. In same content, the results of TSC (imported mutton) were (5.00, 6.97, 5.40, 5.60, and 5.67 log_{10}CFU/g) respectively. Average of TSC (sectors D & E) showed that there are no significant differences (P > 0.05) between local and imported meat (5.77 and 5.57 log_{10}CFU/g) (5.60 and 5.67 log_{10}CFU/g) respectively.

4.4 Salmonella Bacteria

The appearance of salmonella is on differential media. The S. S. Agar media was used to determinate the form of colorless colonies with or without a black center. As well as, an XLD media was used to determinate colonies appear red in a black center or without it. The examination reported that Salmonella was found only in the sector (B).

Table (4) showing the different chemical reactions used in the definition of different types of coliform bacteria in mutton. The result (Table 5) of these tests was to identify the following bacterial species: *E. coli, Enterobacter spp., Citrobacter spp., Klebsiella spp., Proteus spp., Pseudomonas spp., and Salmonella* spp.

Table 4: Biochemical reactions for identification of coliform bacteria in sheep meat [26]

Identification	TSI				SIM			Simmon	VP	MR	Catalase	Oxidase	Gram Stain
	Slant	Bottom	H_2S	Gas	Sulfide	Indole	Motility						
E. coli	A	A	-	+	-	+	+	-	-	+	+	-	-
Enterobacter	A	A	-	-	-	-	+	+	-	+	+	-	-
Citrobacter	Alk /A	A	d	+	d	-	+	+	-	+	+	-	-
Klebsiella	A	A	-	+	-	+	-	+	+	-	+	-	-
Proteus	Alk	A	+	+	+	V	+	d	-	+	+	-	-
Pseudomonas	Alk	Alk	-	-	-	-	+	+	-	-	+	+	-
Salmonella	Alk	A	V	+	V	-	+	-	-	+	+	-	-

Alk: alkaline reaction, A: Acid reaction, butt: at bottom, MR: Methyl Red, VP: Vogas Proskauer,
+: positive result, - : Negative result, d: different results, V: Variable

Table (5) described that the percentage of isolated bacteria of sheep meat in Benghazi market *was E. coli 34.3%, Enterobacter spp. 14.3%, Citrobacter spp. 20%, Klebsiella spp. 5.7%, Proteus spp. 14.3%, Pseudomonas spp. 5.7%, and Salmonella spp. 5.7%.*

Table 5: percentage of isolated bacteria of sheep meat samples in Benghazi market.

Bacteria	Number of samples	percentage
E. coli	12	34.3%
Enterobacter spp.	5	14.3%
Citrobacter spp.	7	20.0%
Klebsiella spp.	2	5.7%
Proteus spp.	5	14.3%
Pseudomonas spp.	2	5.7%
Salmonella spp.	2	5.7%
Total	35	100%

5 Discussion

Bacteria play an important role in meat contamination and disintegration as well as in food poisoning. When the number of bacteria in meat exceeds 10^7 cells, there is evidence of bacterial contamination of the meat. Direct comparison of results is difficult due to differences in the study methodologies, such as the type of slaughtering, improved enrichment and isolation procedures, differences in sample size, the type of sample and how and when it was collected [27]. Borne diseases (*Escherichia coli* O157: H7) from food products of animal origin have become known in human infections [28]. According to the Libyan standard (600/2013), the permitted number of coliform bacteria (*E. coli* O157: H7) and *Salmonella* must be negative samples (0/25 g / sample) as well as *Staphylococcus aureus*. Meat samples in all sections of Benghazi city showed high rates of microbial content. According to the meat production under good health conditions, the samples tested showed that 40% were acceptable and 60% of the samples exceeded the microbial limit. In all sectors, the results (Table 2) showed that the average of *E. coli* was between (4.41 to 7.15 and 4.34 to 6.87 $\log_{10}$CFU/g) in local and imported lamb meat, respectively, which was higher than the permissible limit in Libya (0.00). Almost the same results, the average of *Staphylococcus aureus* was between (5 to 6.87 $\log_{10}$CFU/g) in both local and imported sheep meat. Additionally, *Salmonella* were found by 5.7% of isolates studied, which must be negative.

Our results (Table 4) showed that the most common appearance of coliform bacteria in meat samples was *E. coli* 34.3%, *Citrobacter spp*. 20%, *Proteus spp*. 14.3%, and *Enterobacter spp* 14.3%.

Our results were fairly close to those obtained El Shrek and Ali [14] in Tripoli restaurants, which were 25.9% *Proteus*, 29.6% *Staphylococcus aureus*, 20.3% *E. coli* H7: O157 and 12.9% in *Salmonella*.

Frazier and Westhaff reported that the presence of contaminated and pathogenic bacteria in meat is caused by [29]:

1. Animals are infected by bacteria
2. Slaughtering the *Salmonella*-bearing animal may cause complete contamination of the carcass.
3. Soiled hooves and hair carry large quantities of bacteria from soil, food, barn or water, which are sources of contamination of the surface of the carcass while removing its skin.
4. Keeping the meat with the intestines and the animal's stomach, as well as using the same utensils increases the likelihood of meat contamination.
5. The butcher and open markets may be another source of pollution when the health conditions are not suitable.
6. Poor sanitary procedures, Slaughterhouse and people dealing with animals may be a source of bacterial contamination.

6 Conclusion

The results of the study showed that there are significant differences between the presences of different types of bacteria and between sectors. According to Libyan and FOA standards, the experiment showed that 40% of the samples had acceptable pollution and 60% had no acceptable contamination. Previous evidence refers to poor enforcement of sanitary procedures in slaughterhouses, meat shops, and used tools.

References:

[1] Food and Agriculture Organization of the United Nations. Animal Production and Health. [Online] April 16, 2016. http://www.fao.org/ag/againfo/themes/en/meat/home.html.

[2] Food and Agriculture Organization of the United Nations. Animal Production and Health. [Online] November 25, 2014. http://www.fao.org/ag/againfo/themes/en/meat/background.html.

[3] Magdaa, A. M. A., Siham, E. S., Shuaib, Y. A., and Abdalla, M.A., Assessment of Bacterial Contamination of Sheep Carcasses at Slaughterhouse in Khartoum State, *SUST*

Journal of Science and Technology, Vol 13, No 2, 2012, pp. 68-72.

[4] Brahmbhatt M. N, and Anjaria J. M., Isolation of bacteria from market goat meat and their in vitro antibiotic sensitivity pattern, *Indian Journal of Animal Science*, Vol. 63, 1991, pp. 522-523.

[5] Elhassan, I. M., Abdelgadir A. E., and Ibrahim A. E., Microbiological assessment of mutton intended for export from Elkadaro export slaughter house, Sudan. *African Journal of Microbiology Research*, Vol 5, No 8, 2011, pp. 893-897.

[6] Obeng A. K., Johnson, F. S., and Appenteng S. O., Microbial Quality of Fresh Meat from Retail Outlets in Tolon and Kumbungu Districts of the Northern Region of Ghana, *International Journal of Science and Technology*, Vol 2, No 6, 2013, pp. 423 – 428.

[7] Hajian, S., Rahimi, E., and Mommtaz, H., A 3-year study of Escherichia coli O157:H7 in cattle, camel, sheep, goat, chicken and beef minced meat, *International Conference on Food Engineering and Biotechnology, IACSIT Press, Singapore*, Vol 9, 2011, pp. 162 – 166.

[8] Madden, R. H., Espie, W. E., Moran, L., McBride, J., and Scates, F., Occurrence of E. coli O157:H7, Listeria monocytogenes, Salmonella and Campylobacter spp. on beef carcasses in Northern Ireland, *Meat Sciences*, Vol 58, No 4, 2001, pp. 343–346.

[9] Delhalle, L., de Sadeleer, L., Bollaerts, K., Farnir, F., Saegerman, C. k., Korsak, N., Dewulf, J., De Zutter, L., and Daube, G., Risk factors for salmonella and hygiene indicators in the 10 largest Belgian pig slaughterhouses, *Journal of Food Protection*, Vol 71, No 7, 2008, pp. 1320-1329.

[10] McEvoy, J. M., Sheridan, J. J., Blair, I. S., and McDowell, D. A., Microbial contamination on beef in relation to hygiene assessment based on criteria used in EU Decision 2001/471/EC, *International Journal of Food microbiology*, Vol 92, No 2, 2004, pp. 217 – 225.

[11] Ghafir, Y., China, B., Dierick, K., De Zutter, L., and Daube G., Hygiene indicator microorganisms for elected pathogens on beef pork and poultry meats in Belgium, *Journal of Food Protection*, Vol 71, No 1, 2008, pp. 35 – 45.

[12] Okonko, I. O., Ukut I, O. E., Ikpoh, I. S., Nkang, A. O., Udeze A. O., Babalola, T. A., Mejeha, O. K., and Fajobi, E. A., Assessment of Bacteriological Quality of Fresh Meats Sold In Calabar Metropolis, Nigeria, *Electronic Journal of Environmental, Agricultural and Food Chemistry*, Vol 9, N0 1, 2010, pp. 89-100.

[13] Collins, N. A., and Thato, S., Isolation of enteric bacterial pathogens from raw mincemeat in Mafikeng, North-West Province, *Life Science Journal*, Vol 8, No S2, 2011, pp. 1-7.

[14] El Shrek, Y. M. and Ali, M. R., Microbiological study of spiced chicken burgers in Tripoli City, Libya, *Eastern Mediterranean Health Journal*, Vol 18, No 6, 2012, pp. 653-62.

[15] Musa, Z., Onyilokwu, S. A., Jauro, S., Yakubu, C. and Musa, J. A., Occurrence of Salmonella in ruminants and camel meat in Maiduguri, Nigeria and their antibiotic resistant pattern, *Journal of Advanced Veterinary and Animal Research*, Vol 4, No 3, 2017, pp. 227-233.

[16] Nagarajan, V., Wahab. A., Shivraj, S., and Alex, L., Study of Bacterial Contamination of Raw Meat in Hyderabad, *MOJ Proteomics Bioinform*, Vol 7, No 1, 2018, pp. 46–51.

[17] Panda, A. K., Kumar, A., Thakur, S. D., and Shalmali, Evaluation of Bacteriological Quality of Meat and Meat Products in Palam Valley of North Western Himalayas, *Journal of Veterinary Public Health*, Vol 10, No 1, 2012, pp. 21-25.

[18] Al-Karablieh, I. M., Faydi, Y. R. And Ali-Shtayeh, M. S., Isolation of Salmonella and Escherichia Coli 0157:H7 from Fresh Meat of Turkey and Imported Frozen Cattle Meat with Emphasis on Isolation of Salmonella from Poultry Eggs, DSpace Repository. [Online] 05 30, *An-najah national university*, 2017.

[19] Food Safety and Inspection Services, *Annual Sampling Program Plan*, United States Department of Agriculture, 2015.

[20] Johnson, Ted R., and Christine, L. C., *Laboratory Experiments in Microbiolo*gy, San Francisco, California: Benjamin / Cummings Publishing Company, 1992.

[21] Marshall, R.T. *Standard Method for the Examination of Dairy Products*. Washington-American Public Health Association, 1992.

[22] Bridson, E.Y. *The OXOID Manual*. Hants: Basingstoke, Hampshire - Unipath, 1995.

[23] Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., Kato-Maeda, M., Miller, S., Nadarajan, R., Brodie, E. L., and Lynch, S. V., Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial

pathogens, *PLoS One Journal*, Vol 6, No 10(2), 2015 :e0117617.

[24] Speck Marvin, *Compendium of methods for the microbiological examination of foods*, American public health association, 1984.

[25] Official methods of analysis, Association of official analytical chemist 19th edition, Washington D.C., USA, 2012.

[26] Holt, J. G., Krieg Noel, R., Sneath, P. H. A., Staley, J. T., and Williams, S. T., *Bergy's manual of determinative bacteriology*, [ed.] Hensyl W. R. 9th ed. Baltimore, Maryland USA: Williams & Wilkins., 1994.

[27] Byrne C. M., Erol I., Call J. E., Kaspar C. W., Buege D. R., Hiemke C. J., Fedorka-Cray P. J., Benson A. K., Wallasce F. M., and Luchansky J. B., Characterization of E. coli O157:H7 from downer and healthy dairy cattle in the upper Midwest region of the United States, *Applied and Environmental Microbiology*, Vol 69, No 8, 2003, pp. 4683-4688.

[28] Jo, M., Kim, J.H., Lim, J., Kang, M., Koh, H., Park, Y., Yoon, D., Chae, J., Eo, S., & Lee, J.H. Prevalence and characteristics of Escherichia coli O157 from major food animals in Korea. *International Journal of Food Microbiology*, Vol 15, No 95(1), 2004, pp. 41-49.

[29] Frazier, W.C. and Westhaff, D. C. *Food Microbiology*, 3rd Edition. McGraw-Hill, New York, 1978

Table 4: Biochemical reactions for identification of coliform bacteria in sheep meat [26]

Identification	TSI				SIM			Simmon	VP	MR	Catalase	Oxidase	Gram Stain
	Slant	Bottom	H_2S	Gas	Sulfide	Indole	Motility						
E. coli	A	A	-	+	-	+	+	-	-	+	+	-	-
Enterobacter	A	A	-	-	-	-	+	+	-	+	+	-	-
Citrobacter	Alk/A	A	d	+	d	-	+	+	-	+	+	-	-
Klebsiella	A	A	-	+	-	+	-	+	+	-	+	-	-
Proteus	Alk	A	+	+	+	V	+	d	-	+	+	-	-
Pseudomonas	Alk	Alk	-	-	-	-	+	+	-	-	+	+	-
Salmonella	Alk	A	V	+	V	-	+	-	-	+	+	-	-

Alk: alkaline reaction, A: Acid reaction, butt: at bottom, MR: Methyl Red, VP: Vogas Proskauer, +: positive result, - : Negative result, d: different results, V: Variable