Evaluation of Hygienic status of local Slaughterhouses in Al – Marj, Libya and Its effect on Microbial Load of Meat

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A B S T R A C T

This study was conducted to evaluate the hygienic condition of local slaughterhouse in Al-Marj City, Libya. A total of 120 samples were collected during twice visits per week for 10 weeks including; 40 meat samples of slaughtered sheep, 20 swabs from equipment, 20 water samples, 20 surface swabs (floor and wall swabs) beside 20 hand swabs from slaughterhouse workers. Samples were subjected to bacteriological assessment via determination of aerobic plate count, Enterobacteriaceae count and coliforms count beside isolation of some potential pathogenic bacteria. The recorded results clarified the mean value of aerobic plate count was 9.9×10^6, 9.1×10^6, 4.1×10^6, 9.5×10^8 and 1.5×10^5 cfu/g for the examined samples of meat, equipment, surfaces, water and workers, respectively while the mean value of Enterobacteriaceae count was 0.5×10^2, 1.0×10^2, 3.3×10^2, 1.1×10^2 and 2.5×10^3 cfu/g for the examined samples of meat, equipment, surfaces, water and workers, respectively and the mean value of Enterobacteriaceae count was 3.4×10^3, 3.5×10^3, 4.3×10^4, 2.4×10^3 and 2.5×10^2 cfu/g for the examined samples of meat, equipment, surfaces, water and workers, respectively. In conclusion, to avoid high bacterial load of meat, application of the HACCP system during abattoirs work, educational programs must be applied to the workers as learning of such workers about sources of contamination of meat and personal hygiene to avoid cross contamination.

Keywords: *Local slaughterhouses, Microbial, Evaluation*

1. Introduction

One of the major and expensive sources of animal protein is meat. Its high nutritive values make it an excellent media for bacterial growth. To ensure production of meat of good keeping quality, slaughtering should be in slaughterhouses under veterinary supervision and complete hygienic measures (Zailani et al., 2016). Microbial contamination of the surfaces of the animal carcasses is mainly due to the existence of a wide range of microorganisms in the environment of the meat processing plants, slaughterhouses and butcher-shops. The main contamination sources of meat occurred during slaughtering processes such as hides and gastrointestinal tract contents of the slaughtered animals, the staff and the work environment. Additionally, carcasses can be contaminated during the slaughter process through the contact with the animal's skin, blood, hair, limbs, bile and stomach, gut contents, or/and facilities, equipment, water supplies, air pollution and worker's hands and clothes (Muhammad et al., 2012).

The sources of microbial contamination of animal carcasses include but not limited to the animal itself and carcass-contact surfaces such as butcher hands, knives, cutting boards, walls, floors, air and water (Darwish et al., 2016).

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There are many microbial indicators for the hygienic measures of the meat-processing and handling plants which include total bacterial count (TBC), Enterobacteriaceae counts (EC), most probable number (MPN) of coliforms, Staphylococcus aureus counts (TSC), mould counts and yeast counts. These indicators give a clear image about the hygienic practices and measures adopted during carcass handling and processing and finally effect on the production of meat of high keeping quality (Mossel et al., 1995). The routine veterinary inspection in the slaughterhouses is not included a microbiological examination. Therefore, microbial contamination of meat may affect its quality with a potential of food poisoning or spoilage due to microbial feeding on meat nutrients such as sugars and free amino acids, which liberate undesired volatile metabolites (Bogere and Baluka, 2014). Contamination of the meat surface with different organisms play an important role in grading and classification of the meat in the world market as future grading schemes which measure both carcass yield and eating quality have the potential to underpin the development and implementation of transparent value-based payment systems which will encourage improved production efficiency throughout the supply chain (Polkinghorne and Thompson, 2010). Local slaughterhouses are suffering from many administrative limitations. There is no penalty enforced the veterinary service authorities in case of fault operations during meat processing that could affect the quality or safety of produced meat. The presence of both un-skinned and skinned carcasses in the same area might be a source of meat contamination by many pathogenic agents (Hemmat et al., 2014). The aims of this study were to evaluate hygienic status of local slaughterhouse in Al Marj, Libya and its impact on microbial load of meat.

Material and methods

2.1. Study area:

This study was conducted to evaluate the hygienic condition of local slaughterhouse in Al Marj City, Libya. It is manually operated slaughterhouse. It is well constructed with a fence and consisted of a slaughtering hall, two quarantine partitions, two eviscerated rooms, emergency slaughtering room and condemnation room. Slaughtering capacity is around 300 heads of sheep per week with fewer slaughtering rate of cattle. The slaughter operations were started early morning usually at 6:00 am and lasted in 10:00 to 12:00 based on the number of heads admitted for slaughtering. The slaughtering area routinely cleaned by tap water at the end of working day. No disinfection programs were applied.

2.2. Sampling:

Samples were collected during twice visits per week for 10 weeks. A total of 120 samples as following: 40 slaughtered meat samples of sheep each about 100 ±10 g, 20 swabs from the used equipment, 20 water samples each sample about 0.5 liter, 20 surface swabs (floor and wall swabs) beside 20 hand swabs from slaughterhouse workers in sterile buffered peptone water. Samples were labeled and transferred in an ice box to the Microbiological Laboratory for bacteriological assessment.

Table (1): Statistical analytical results of aerobic plate count of different examined samples

Samples	Minimum	Maximum	Mean ± S.E.M
Meat	0.8×10^6	7.3×10^7	$9.9 \times 10^6 \pm 1.14 \times 10^5$
Equipment	1.0×10^5	8.2×10^7	$9.1 \times 10^6 \pm 1.15 \times 10^5$
Surfaces	0.5×10^5	3.2×10^7	$4.1 \times 10^6 \pm 1.11 \times 10^4$
Water	2.5×10^6	9.6×10^9	$9.5 \times 10^8 \pm 1.17 \times 10^5$
Workers	0.1×10^4	1.4×10^6	$1.5 \times 10^5 \pm 0.14 \times 10^5$

Table (2): Statistical analytical results of Enterobacteriaceae count of examined samples

Samples	Minimum	Maximum	Mean ± S.E.M
Meat	1.5×10	2.5×10^2	$0.5 \times 10^2 \pm 0.03 \times 10$
Equipment	0.5×10	4.3×10^2	$1.0 \times 10^2 \pm 0.34 \times 10$
Surfaces	1.6×10	5.7×10^2	$3.3 \times 10^2 \pm 1.66 \times 10$
Water	0.6×10	2.0×10^2	$1.1 \times 10^2 \pm 0.11 \times 10^2$
workers	0.5×10	2.5×10^2	$2.5 \times 10^3 \pm 1.11 \times 10^2$

Table (3): Statistical analytical results of coliforms count of examined samples

Samples	Minimum	Maximum	Mean ± S.E.M
Meat	2.6×10^2	2.7×10^4	$3.4 \times 10^3 \pm 2.66 \times 10^2$
Equipment	1.5×10^3	4.3×10^4	$3.5 \times 10^3 \pm 2.34 \times 10^2$
Surfaces	2.6×10^3	5.7×10^4	$4.3 \times 10^4 \pm 3.66 \times 10^2$
Water	2.6×10	2.7×10^3	$2.4 \times 10^3 \pm 1.66 \times 10$
Workers	0.8×10	4.3×10^2	$2.5 \times 10^2 \pm 1.33 \times 10$

Table (4): Results of microbiological quality of meat samples matched to standard limits

Bacterial counts	Samples within permissible limits		Samples exceeding permissible limits	
	Positive	%	Positive	%
Aerobic plate count	35	87.5	5	12.5
Enterobacteriaceae count	19	47.5	21	52.5
Coliforms count	25	62.5	15	37.5

Acceptability was according to **International Organization for Standardization, (2008).**

APC must not exceed 10^6 CFU/g.

There was no permissible limit for Enterobacteriaceae.

Coliforms must not exceed 10^2 CFU/g

Table (5): Prevalence of some potential pathogenic bacteria in different examined samples

Bacteria / Samples	No. of samples	E. coli		Salmonellae		Staphylococcus aureus	
		Positive	%	Positive	%	Positive	%
Meat	40	11	27.5	1	2.5	2	5.0
Equipment	20	6	30.0	0	0.0	1	5.0
Surfaces	20	3	15.0	0	0.0	1	5.0
Water	20	3	15.0	0	0.0	0	0.0
Workers	20	4	20.0	2	10.0	3	15.0
Total	120	27	22.5	3	2.5	7	5.8

Fig. (1): Showing the slaughtering hall where slaughtering processes including; bleeding and skinning were done manually on the floor with using tap water.

Fig. (2): Showing the out construction of slaughterhouse and water tanks reflecting low quality infrastructure

2.3. Samples Preparation:
2.3.1. Meat samples:
It was performed according to APHA, (2002). Upon received to the laboratory, 25 g from each meat sample was aseptically incised with sterile scalpel, and diluted with 225 ml of sterile 1% peptone water (w/v) in sterile stomacher bag and homogenized in a Stomacher (Lab-blender, 400) for 1 minute providing 10- 1 dilution. Tenfold serial dilution was prepared up to 10-6.
2.3.2. Equipment, floor and wall swabs:
A total of 40 swabs were collected from equipment, floors and walls using sterilized cotton swabs by swabbing on the surface of floor and walls in approximately 1 cm2 surface area, and then insert the swabs in sterile peptone water (Merck) and transport under the chilling condition to the laboratory.
2.3.3. Water samples:
Water samples were collected from the identified functional tanks and water taps. Samples were labeled and transported in coolers to the laboratory with minimal delay.
2.3.4. Workers samples:
A total of 20 hand swabs were collected from workers in cattle and poultry farm. They were obtained in sterile swab placed in a sterile test tube containing buffer peptone water and each tube was covered with sterile cotton plug then they were transferred with a minimum of delay in an ice box to the laboratory. On arrival they were incubated for 12 hours.
2.4. Microbiological evaluation of the prepared samples:
2.4.1. Determination of aerobic plate count (ISO 4833: 2003).
2.4.2. Determination of Enterobacteriaceae count (ISO 4833: 2003).
2.4.3. Determination of coliforms counts (ISO 4833:2003).
2.4.5. Detection of Salmonellae (ISO 6579-1/2017).
2.4.6. Detection of E. coli (ISO, 7251: 2005).
2.4.6. Detection of Staphylococcus aureus (ISO 4833: 2003).

Results

The recorded data in Table (1) showed that the mean value of aerobic plate count was 9.9×10^6, 9.1×10^6, 4.1×10^6, 9.5×10^8 and 1.5×10^5 cfu/g for the examined samples of meat, equipment, surfaces, water and workers, respectively. The recorded data in Table (2) showed that the mean value of Enterobacteriaceae count was 0.5×10^2, 1.0×10^2, 3.3×10^2, 1.1×10^2 and 2.5×10^3 cfu/g for the examined samples of meat, equipment, surfaces, water and workers, respectively. The recorded data in Table (3) showed that the mean value of Enterobacteriaceae count was 3.4×10^3, 3.5×10^3, 4.3×10^4, 2.4×10^3 and 2.5×10^2 cfu/g for the examined samples of meat, equipment, surfaces, water and workers, respectively. The presented data in Table (4) showed the percentage of meat samples matched to standard limits based on different bacterial counts. It was recorded that 87.5%, 47.5% and 62.5 % of meat samples were comply with the standard limits of Aerobic plate count, Enterobacteriaceae count and Coliforms count. The presented data in Table (5) showed that the prevalence of E. coli was 27.5, 30, 15, 15 and 20 % in the examined samples of meat, equipment, surfaces, water and workers, respectively while the prevalence of Salmonellae was 2.5 and 10% in the examined samples of meat and workers, respectively. Finally, the prevalence of Staphylococcus aureus was 5, 5, 5, 0.0 and 15% in the examined samples of meat, equipment, surfaces and workers, respectively.

Discussion

Food-borne illnesses resulted from contaminated meat consumption with pathogenic bacteria such as Salmonella spp., S. aureus and E. coli which adversely affects shelf-life and renders the meat unfit for human consumption to avoid its several human health hazardous which ranging from mild illness to death (Bogere and Baluka, 2014).
Bacterial count of perishable food is used to evaluate its quality and shelf-life. However, high count may be attributed to unsanitary methods of slaughter or exposure to conditions favoring bacterial proliferation (Sharma et al., 1996).
The recorded data in Table (1) showed that the mean value of aerobic plate count was 9.9×10^6, 9.1×10^6, 4.1×10^6, 9.5×10^8 and 1.5×10^5 cfu/g for the examined samples of meat, equipment, surfaces, water and workers, respectively.
Enterobacteriaceae group of bacteria is the most challenging bacterial contaminant to raw and processed meat products worldwide. Salmonella and E. coli are the most predominant species in all food poisoning cases associated with meat consumption (Al-Mutairi, 2011). The occurrence of Enterobacteriaceae indicated microbiological and toxigenic bacteria in meat and lead to public health hazard. Also, they indicated poor sanitary conditions during slaughtering, handling and preparation (Mira, 1989) and it is used as an indicator of fecal contamination of fresh meat carcasses. Also, Enterobacteriaceae count has been recommended as an indicator of the contamination by intestinal material, therefore their presence in high number indicates inadequacy of general hygiene in food plant. Moreover,

many species cause spoilage and deterioration of meat (Banwart, 1989).
The recorded data in Table (2) showed that the mean value of Enterobacteriaceae count was 0.5×10^2, 1.0×10^2, 3.3×10^2, 1.1×10^2 and 2.5×10^3 cfu/g for the examined samples of meat, equipment, surfaces, water and workers, respectively.
It was clear from the recorded results that Enterobacteriaceae count seemed to be high that it should draw the attention to the contamination from enteric sources. The higher levels of Enterobacteriaceae count after evisceration may be attributed to occasional rupture of viscera resulting in spread of gut contents on to the carcass (Viscera could be considered a potential source of contamination unless it was removed intact). Also, it may be attributed to the slaughtering practices that were followed including; the same workers performed all of the slaughtering practices like removal of skin, evisceration and cutting using the same knives for all operations leading to spreading contamination (Grau, 1986).
Coliforms are Gram negative indicator groups of bacteria which widely used as a measure of the hygienic characteristics of food. They have the advantage of being enumerated inexpensively and easily for quantifying the performance of a production process, when particular pathogens or spoilage organisms might difficult to detect (Jordan et al., 2007).
The recorded data in Table (3) showed that the mean value of Enterobacteriaceae count was 3.4×10^3, 3.5×10^3, 4.3×10^4, 2.4×10^3 and 2.5×10^2 cfu/g for the examined samples of meat, equipment, surfaces, water and workers, respectively.
Presence of coliforms in meat has an epidemiological interest as some of its members were pathogenic, and may result in serious infections and food poisoning. Thus, the total coliforms count may be used as broad base indicating fecal contamination of meat due to inadequate processing and/or post processing contamination of meat ICMSF, (1998).
Coliforms were the prevalent bacteria on the beef meat at the abattoir and have been isolated from different sites in variable number. Meanwhile the occurrence of large number of coliforms on meat surface is highly undesirable (Mira, 1989). The occurrence of high number of coliforms on the meat surfaces is important in reflecting the hygienic quality of meat and the test for coliforms bacilli is considered of much greater value in assessing its quality (Cruickshank et al., 1975)
The presented data in Table (4) showed the percentage of meat samples matched to standard limits based on different bacterial counts. It was recorded that 87.5%, 47.5% and 62.5 % of meat samples were comply with the standard limits of Aerobic plate count, Enterobacteriaceae count and Coliforms count.
Generally, E. coli is the most common organism in the intestinal tract of human and animals. It has a traditional role in food and water microbiology as an index of faecal contamination. The presence of E .coli in food is mainly associated with outbreak of gastroenteritis syndromes (Varman and Evans, 1991). E. coli was considered one of the important causes of febrile types of gastroenteritis transmitted by foods (Mossel et al., 1995).
Salmonella are the most complex of all the Enterobacteriaceae and have more than 2200 serotypes (Ewing , 1986) and it could be considered as one of the most causes of food borne illness since the major source of human illness is the contaminated carcasses (Small et al., 2006).
The presented data in Table (5) showed that the prevalence of E. coli was 27.5, 30, 15, 15 and 20 % in the examined samples of meat, equipment, surfaces, water and workers, respectively while the prevalence of Salmonellae was 2.5 and 10% in the examined samples of meat and workers, respectively. Finally, the prevalence of Staphylococcus aureus was 5, 5, 5, 0.0 and 15% in the examined samples of meat, equipment, surfaces and workers, respectively
Obtained results similar to Holeckova et. al., (2002) and Olayinka & Sani, (2014), whereas, higher results were found by Hemmat et al., (2014) who tested quality of beef and edible offal at abattoir level in governorate, they reported 2.36×10^5, 10.8×10^4, 44×10^3 cfu/g for total aerobic bacteria, E. coli and S. aureus, respectively. Furthermore, Aftab et al. (2012) and Bogere & Baluka, (2014) recorded higher results in Uganda Abattoir meat as following; were 1.64×10^9, 8.4×10^4, 2.7×10^3 cfu/g of the total bacterial count, E. coli and S. aureus respectively. Hughes et al., (2015) recorded higher results in Ghana slaughtered meat; 8.32, 5.97, 5.50 (log10cfu/g), Total viable mean counts, E. coli, S. aureus respectively.
The slaughterhouse may be the microbial source of meat contamination in case of bad hygienic conditions. Studying of the microbial quality of the slaughterhouse and meat reflects the hygienic quality in the slaughterhouses and estimates the meat quality and the public health risk of food poisoning bacteria. The slaughterhouses should have adequate clean water (free from chemicals or high microbial load). Abattoir requires about 149,358 liters of water for the cleaning and slaughter process (Gracey et. al., 1999). Unhygienic disposal of abattoir waste may contaminate ground water (Adebowale et. al., 2010).
The results of microbial quality of floor and walls agreed with a study prepared by Gill & McGinnis, (1999) whereas, higher results obtained by Paulsen et al., (2011) who investigated microbial loads on meats and swabs from slaughterhouse and reported the microbial load exceeds 5 logs10 cfu, and become unacceptable on food.
Figure (1) showed the slaughtering steps slaughterhouse hall slaughtering processes, bleeding and skinning which occur on the floor, different species slaughtered in the same area using tap water for cleaning. According to Libyan regulations, slaughtering must be occurred during complete animal consciousness by cutting the two jaguar vein which called Halal (Islamic)

slaughtering, which is aimed to have the meat of good public health and avoid many zoonotic diseases like Salmonellosis, E. coli and Staphylococci infections (Roberts, 2011). Slaughtering, skinning, and evisceration on the ground without separation between dirty and clean area lead to high possibilities of cross-contamination during meat processing which poses hazards of meat consumers of foodborne illness. Other important possibilities for the high microbial load of meat were the dirty hands and clothes of workers and the absence of any written sanitary measures on the slaughterhouse, lack of workers training for these measures. Therefore, all sanitary measures in the slaughterhouse should be applied and regularly evaluated to ensure quality control. There is no data on contagious or infectious diseases detected in the slaughterhouse.

Conclusion

In conclusion, to avoid high bacterial load of meat, application of the HACCP system during abattoirs work, educational programs must be applied to the workers as learning of such workers about sources of contamination of meat and personal hygiene to avoid cross contamination.

Author contributions

All authors contributed to conception and realization of the work. All the authors have contributed to the paper redaction and given their approval to the final version of the manuscript.

Conflict of interests

The authors have not declared any conflict of interests.

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