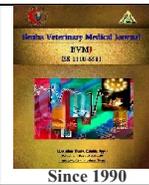




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### Original Paper

## Prevalence of some bacterial contamination during processing of imported and local natural casing at a plant in Cairo governorate

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### ABSTRACT

A total of 90 random samples represented by local and imported natural sheep Casing (45 of each) were collected during certain processing steps from a plant which is working in processing natural casing and implementing HACCP system at Cairo governorate. These samples were subjected to some bacteriological examination as *Aerobic Plate Count (APC)*, *Enterobacteriaceae*, *Coliforms* and *Staphylococci* counts. The results revealed that imported samples showed relatively higher mean values than the local one; where APC was ( $5.47 \pm 0.20, 5.51 \pm 0.18, 5.56 \pm 0.22$ ), *Enterobacteriaceae* were ( $1.41 \pm 0.14, 1.99 \pm 0.23, 1.81 \pm 0.27$ ), *Coliforms* were ( $1.42 \pm 0.22, 0.98 \pm 0.21, 0.92 \pm 0.26$ ) and *Staphylococci* were ( $3.16 \pm 0.17, 3.21 \pm 0.15, 3.16 \pm 0.12$ ) log CFU/g for imported samples at initial Salting, Sorting, Final Salting Steps, respectively. While, *APC*, *Enterobacteriaceae*, *Coliforms* and *Staphylococci* were ( $5.48 \pm 0.13, 5.62 \pm 0.14, 5.47 \pm 0.12$ ), ( $1.00 \pm 0.00, 1.95 \pm 0.19, 1.23 \pm 0.12$ ), ( $0.83 \pm 0.13, 1.25 \pm 0.21, 0.54 \pm 0.07$ ) and ( $3.09 \pm 0.17, 3.06 \pm 0.15, 2.71 \pm 0.16$ ) log CFU/g for local samples at initial Salting, Sorting, Final Salting Steps, respectively. Consequently, strict monitoring of good practices during processing, maintaining the cold chain during transportation and storage of raw materials reduce the bacterial load as well as make the casings safer. Finally, the present study proved that natural casing is considered of public health hazard if not treated carefully.

## 1. INTRODUCTION

Due to behavioral and economic reasons, large quantities of animals' offal especially intestine are eaten by the people all over the world, especially in developed countries. The high popularity of animal offal as a food of high protein contents worldwide was highlighted, however very rare studies focused on evaluating the microbial quality of diverse edible offal especially bacterial contaminants foodborne that have public health significance.

The raw materials including meat and natural casings are the main vehicles for food-borne pathogens and contaminants in sausage production (Vignolo et al., 2010). The growth in the consumption of animal by-products over the past decades due to their ability to combat protein malnutrition and food insecurity in many developing countries (Alao et al., 2017).

The intestines of sheep, cattle, and hogs are the longest and oldest established form of processed meat which so-called "sausage", it has been used for thousands of years (Wijnker et al., 2006). Natural casings are produced from the intestines of different species and used as edible containers for many different types of sausage around the world. An animal intestine incorporates a wide range of enteric bacteria which are capable of causing food poisoning in human upon consumption (Wijnker, 2009).

Poor hygienic practices in meat processing plants may result in the contamination of meat and meat products with

pathogens causing a serious risk for human health (Yang et al., 2012).

High Aerobic Plate Count may be attributed to the contamination of the product from different sources or unsatisfactory processing as well as unsuitable condition during storage (Zaharan-Dalia, 2008).

*Staphylococci* can contaminate foods and cause illness in humans when ingested, so it is frequently implicated in food borne illness (Prange et al., 2005).

High Coliforms count indicates poor hygienic quality of meat and may be responsible for economic losses and presence of enteric pathogens which constitute public health hazards (Yadav et al., 2006).

*Enterobacteriaceae* can be regarded as indicator and index organisms as there are close correlation existing between the *Enterobacteriaceae* counts and extend of fecal pollution (Hayes, 1992).

Therefore, the present study is aimed to make a comparison between the hygienic status of local sheep casing and imported one through the following points: Aerobic plate count, *Enterobacteriaceae* count, *Coliforms* count and *Staphylococci* count.

## 2. MATERIAL AND METHODS

### 2.1. Samples collection:

A total of 90 random samples of local and imported natural sheep casing (45 for each), were collected from certain three steps (Initial Salting, Sorting, Final Salting) which obtained

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from a plant work on natural casing manufacture at Cairo governorate to evaluate the bacterial status of the casings.

### 2.2. Bacteriological examination:

1. Preparation of samples according to (ISO 6887-2/2003).

A homogenized preparation for bacteriological determinations was prepared by mixing 10g of representative samples in a stomacher bag with 90 ml of peptone water 0.1% (diluent) then stomached until a homogenate suspension to provide a dilution of  $10^{-1}$  then tenth fold serial dilutions were prepared up to  $10^{-4}$ .

2. Aerobic plate count was detected by spreading technique according to (APHA, 2001). Using PCA incubated at 37°C for 48 hrs.

3. *Enterobacteriaceae* count according to (ISO 21528 - 2/2004).

Using Violet red bile glucose agar incubated at 37°C for 24hrs.  $\pm$  2hrs. Colonies showed purplish-red colonies surrounded by a red zone of precipitated bile acid.

4. Enumeration of *Coliforms* bacteria by most probable number (MPN) according to (FDA, 2005). Using lauryl sulphate tryptose broth tubes incubated for 24h.  $\pm$  2h for gas production at 30°C or 37°C.

5. Determination of total *Staphylococci* count according to (USDA, 2011) using Baird Parker agar incubated at 37°C for 48 hrs. Colonies are Shiny black.

### 2.3 Statistical analysis:

Means of examined samples were compared by using procedure from SPSS software (release 20, IBM CO) after logarithmic transformation for bacteriological counts. Significance was tested using paired samples T-test at  $\alpha = 0.05$ .

## 3. RESULTS

The results of the examined natural sheep casing (local and imported) are presented in Tables (1-4) and Figures (1-4). According to the results of the present study, *Aerobic platelet count* (APC), and *Enterobacteriaceae*, *Coliform* and *Staphylococci* are prevalent in both local and imported natural casing samples. Both *Enterobacteriaceae* and *Staphylococci* counts of the local sample were lower than those of the imported samples especially at final salting step. The same for *Coliform* except at the sorting step, where the imported samples were the lowest one. Meanwhile, *APC* of the local samples were higher than those of the imported samples except at the final salting step, where the imported samples were the highest one.

In general, the imported samples showed bacterial counts slightly higher than the local one. There is no significant difference between the two means of imported and local examined samples except in case of initial salting step for both *Enterobacteriaceae* and coliforms and final salting step for *Staphylococci*.

Table 1 Aerobic plate count (log CFU\*/g) of examined samples of natural sheep casing (n=15 of each step)

Steps	Imported				Accepted Samples		Local				Accepted Samples	
	Minimum	Maximum	Mean	SE*	No.	%	Minimum	Maximum	Mean	SE*	No.	%
Initial Salting	4	6.54	5.47a*	0.2	6	33	4.6	6.48	5.48a*	0.13	3	20
Sorting	4	6.6	5.5 a*	0.18	4	27	4.7	6.3	5.62a*	0.14	2	13
Final Salting	4	6.78	5.56a*	0.22	5	33	4.6	6.26	5.47a*	0.12	5	33

Table 2 *Enterobacteriaceae* count (log CFU\*/g) of examined Samples of natural sheep casing (n=15 of each step).

Steps	Imported				Accepted Samples		Local				Accepted Samples	
	Minimum	Maximum	Mean	SE*	No.	%	Minimum	Maximum	Mean	SE*	No.	%
Initial Salting	1	2.68	1.41 a*	0.14	13	87	1	1	1.00 b*	0	15	100
Sorting	1	3.48	1.99 a*	0.23	10	67	1	3.11	1.95 a*	0.19	10	67
Final Salting	1	3.56	1.81 a*	0.27	10	67	1	2.32	1.23 a*	0.12	13	87

Means higher or lower level than reference value. \* N.B

Table 3 *Staphylococci* count (log CFU\*/g) of examined samples of natural sheep casing (n=15 of each step).

Steps	Imported				Local			
	Minimum	Maximum	Mean	SE	Minimum	Maximum	Mean	SE
Initial Salting	1	4	3.16 a*	0.17	2	4.11	3.09 a*	0.17
Sorting	2.48	4.48	3.21 a*	0.15	2.2	3.76	3.06 a*	0.15
Final Salting	2.6	3.9	3.16 b*	0.12	1.85	4.26	2.71 a*	0.16

Table 4 *Coliform* count (log CFU\*/g) of examined Samples of natural sheep casing (n=15 of each step).

Steps	Imported				Local			
	Minimum	Maximum	Mean	SE	Minimum	Maximum	Mean	SE
Initial Salting	0.48	2.66	1.42 a*	0.22	0.48	2.32	0.83 b*	0.13
Sorting	0.04	2.66	0.98 a*	0.21	0.04	2.66	1.25 a*	0.21
Final Salting	0.04	2.66	0.92 a*	0.26	0.04	1.45	0.54 a*	0.07

There is significant difference between means having different letters in the same raw when ( $P \leq 0.05$ ).  
SE\*standard error of mean.  
CFU\*Colony Forming Unit.

#### 4. DISCUSSION

In this investigation, the bacterial contamination contents for both local and imported sheep casings have been studied and compared. The bacteriological evaluation and detection of *Aerobic platelet count (APC)*, *Enterobacteriaceae*, *coliforms*, *Staphylococci* have been studied.

The total aerobic plate count reflects the bacterial contamination and declared the hygienic quality of both raw meat and meat products. Meanwhile, *Coliform* counts may indicate faecal contamination either from human or animal sources and its presence indicate poor sanitation and handling (Paulsen et al., 2006).

The results illustrated in table (1) revealed that the mean value of *APC* in the final salting step of the local casing samples was 5.47 log (cfu/g), with a minimum and the maximum value of 4.60 and 6.26 log (cfu/g). These results are similar to those of El-khateib (1997) and Al-Mutairi (2011), but it is higher than those of Shaltout et al. (2016). This mean value is lower than those of Yassien et al. (2007) and El-baz et al. (2016). On the other hand, the mean value of *APC* in the final salting step of the imported casing samples was 5.56 log (cfu/g), with a minimum and the maximum value of 4.00 and 6.78 log (cfu/g), respectively. These results are similar to those of Oluwafemi and Simisaye (2006).

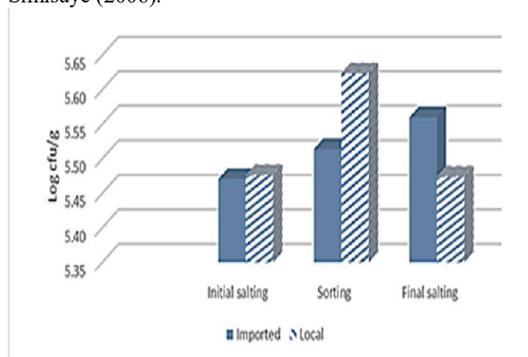


Figure 1 Mean of aerobic plate count of examined samples (log cfu/g) during 3 steps of processing of sheep casing.

*Enterobacteriaceae* count has an epidemiological importance as some of its members are pathogenic and may cause serious infections and food poisoning. They occur as normal flora of the intestinal tract. They are widely distributed in nature. Their presence in large numbers in food indicates inadequate processing/or recontamination due to cross contamination by raw materials, dirty equipment or poor hygienic handling (Ikeme, 1990). The results illustrated in table (2) revealed that the mean value of

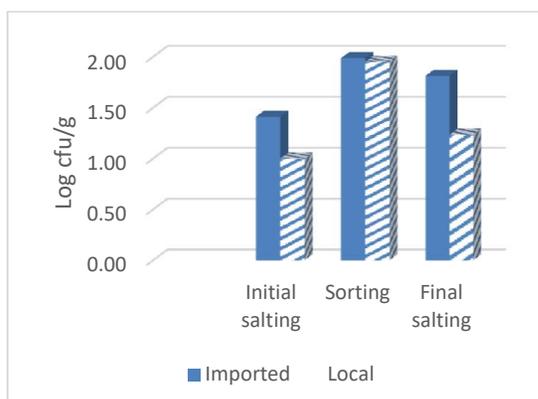


Figure 2 Mean of Enterobacteriaceae count of examined samples (log cfu/g) during 3 steps of processing of sheep casing.

*Enterobacteriaceae* count in the final salting step of the local casing samples was 1.23 log (cfu/g), with a minimum and the maximum value of 1.00 and 2.32 log (cfu/g). These results are similar to those obtained by Shaltout et al. (2016) and lower than those of Al-Mutairi (2011), Yassien et al. (2007) and El-khateib (1997).

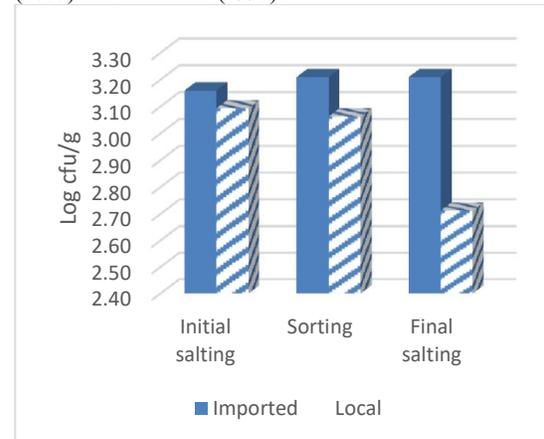


Figure 3 Mean of Staphylococci count of examined samples (log cfu/g) during 3 steps of processing of sheep casing.

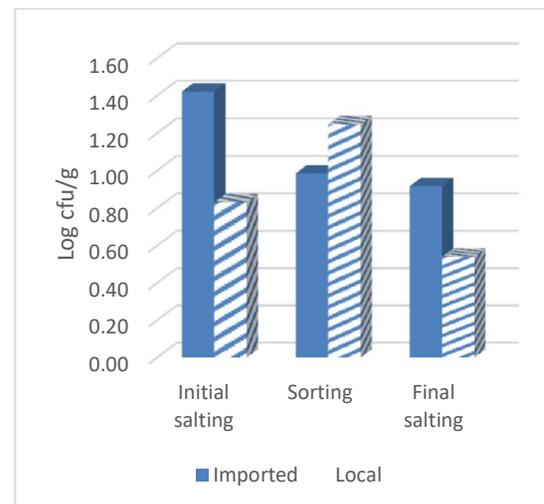


Figure 4 Mean of Coliforms count of examined samples (log cfu/g) during 3 steps of processing of sheep casing.

While the mean value of *Enterobacteriaceae* count in the final salting step of the imported casing samples was 1.81 log (cfu/g), with a minimum and the maximum value of 1.00 and 3.56 log (cfu/g), respectively. Which were lower than those of González et Diez (2002) and Tabanelli et al. (2012) and similar to those obtained by Pedonese et al. (2020). *Enterobacteriaceae* are useful indicators of hygiene and post processing contamination of processed foods (El-Mutiri, 2011).

*Staphylococci* produce some enzymes which are implicated with *Staphylococcus* invasiveness and many extracellular substances some of which are heat stable enterotoxins that renders the food dangerous even though it appears normal and extensive cooking can kill the bacteria, but the toxins may not be destroyed because most of them are gene based i.e. they can be carried on the plasmid (Prescott et al., 2005). The Staphylococcal enterotoxins (SEs) are responsible for the symptoms. The disease is characterized by symptoms including nausea, vomiting, abdominal cramps and diarrhea lasting from 24 to 48 h and the complete recovery usually

occurs within 1-3 days (Llewelyn and Cohen, 2002). The results illustrated in table (3) revealed that the minimum and the maximum value of *Staphylococci* count in the final salting step of the local casing samples were 1.85 and 4.26 log (cfu/g), respectively, with a mean value of 2.71 log (cfu/g). The minimum value result agreed with those obtained by Shaltout et al. (2016) results, but the maximum value in our results is higher than Shaltout et al. (2016) results. Also, the mean value of *Staphylococci* count of the local casing samples is higher than those reported by Younis et al. (2019), Shaltout et al. (2016) and Al-Mutairi (2011). On the other hand, the minimum and the maximum value of *Staphylococci* count in the final salting step of the imported casing samples were 2.60 and 3.90 log (cfu/g), respectively, with a mean value of 3.21 log (cfu/g) which is lower than those obtained by Tabanelli et al. (2012), Soyer et al. (2005) and Chawla et al. (2006).

The results illustrated in table (4) revealed that the mean value of *Coliform* count in the final salting step of the local casing samples was 0.54 log (cfu/g), with a minimum and the maximum value of 0.04 and 1.45 log (cfu/g). These results are lower than those of Shaltout et al. (2016). While the mean value of the *Coliform* count in the final salting step of the imported casing samples was 0.92 log (cfu/g), with a minimum and the maximum value of 0.04 and 2.66 log (cfu/g). The mean value is lower than those of Chawla et al. (2006). They are indicators of fecal pollution at slaughterhouse which begin from skinning and direct contact with knives and worker's hands. Also, during evisceration and washing, contamination may come from intestinal contents as well as from water during rinsing and washing of carcasses.

The increase of bacterial counts may be due to prolongation storage or fluctuation in temperature during storage too, as well as poor personal hygiene of the workers or handlers during transporting and processing of those casings.

## 5. CONCLUSION

Natural casing is considered as a good medium for the growth of different bacterial species and consequently, presence of public health hazards. The presence of this bacterial species in the final product, especially beyond permissible limits may be indicate its' contamination before, during and even after the processing, distribution and storage of the casings also, indicate lack of hygienic control during the processing steps. This lately reflects on consumers' health and safety. Therefore, there is a need for determination of a specific standards and shelf life for natural Casings (i.e., Egyptian food legislation for natural Casing). So, the researcher recommended that: periodical inspection on the similar plants to be sure that HACCP system was verified, and its' employees are well trained, in addition to their health certificates are valid. Consequently, strict monitoring of good practices during processing, maintaining the cold chain during transportation and storage of raw materials. Finally, the present study proved that natural casing is considered of public health hazard if not treated carefully.

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