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Microbial Profile of Imported Carcass under Chilled Storage

Esraa F. Hussein¹, Ali M. Ahmed^{1*}, Hanan A. Elghayaty², Heba M. Shaheen¹

¹Department of Food Hygiene, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

²Department of Food Hygiene, Animal Health Research Institute Port-Said, A.R.C., Egypt.

*Correspondence

Ali Meawad Ahmed Department of Food Hygiene, Faculty of Veteri-

nary Medicine, Suez Canal University, Ismailia, Egypt. E-mail address: ameawad@yahoo.com

Abstract

Bacterial contamination has been proven to be common in a variety of foods, especially meats. For this reason, this study was conducted to evaluate the bacteriological quality of imported chilled meat traded in Port-Said markets where 64 random samples of chilled meats represented by 28 imported chilled beef meat samples from lots arrived at Port-Said governorate (marketed) and 36 imported chilled beef meat samples collected from retailed markets at Port-Said governorate (marketed). Samples were analyzed for their total aerobic count, *Enterobacteriaceae* count, *E. coli*, total *Staphylococcus*, and *S. aureus* counts and detection of *Salmonellae*. The total bacterial count recorded an average of 10.73x10⁴ and 2.5x10⁶ in un-marketed and marketed chilled meat respectively. The results showed that 18 out of 64 meat samples were positive for *Enterobacteriaceae* and 6 samples out of them were unaccepted for human consumption. The incidence of *E. coli* was in 6 samples from the examined chilled samples, and the 6 were unaccepted. For *Staphylococcus*, there were 24 positive and 4 samples were unaccepted for consumption. Two samples out of 64 samples were positive for *Salmonella* and considered unfit for human consumption. The obtained results confirmed the poor bacteriological quality of some imported chilled meat that is marketed in Port-Said retailed markets which is related to unhygienic transportation methods until reach the retailed markets.

KEYWORDS Enterobacteriaceae, carcasses, E. coli, meat, S. aureus, Salmonella

INTRODUCTION

Meat is a significant food source for humans, supplying many essential nutrients. Its demand is particularly high in developing countries (Juarez *et al.*, 2021). Meat is considered a high-risk food due to its abundant nutrients that could favor the growth of microorganisms and the carriage of pathogenic microorganisms due to its high level of moisture, high percentage of nitrogenous compounds, good supply of minerals, glycogen, and its favorable pH which are an appropriate environment for different types of microbial growth (Bosilevac *et al.*, 2019). Generally, a great diversity of microorganisms inhabits fresh meat, but different types may become dominant, which depends on many factors like pH, textures, composition, storage temperature, and transportation means of raw meat (Adu-Gyamfi *et al.*, 2012).

Foodborne diseases are the major cause of mortality and infectious diseases, especially in developing countries. A variety of pathogenic microorganisms including bacteria, viruses, protozoans, and parasites are involved in several severe outbreaks worldwide (WHO, 2016). Most *E. coli, Staphylococcus* spp., and *Salmonella* spp. were found to be the cause of serious foodborne diseases (Bhandare *et al.*, 2017). Sanitary conditions of the

slaughterhouses, handling of meat, butcher shops, preservation, improper packing, selling process of meat, and the environmental conditions play great roles in the level of contamination (Bhandare *et al.*, 2017). Meat tissues get contaminated during the various stages of slaughter, handling, transportation, and preservation. The risk of contamination happens from the point of entry of the animal into the slaughterhouse up to the time of meat consumption. In this regard, abattoir environments and slaughter processes play a leading role in the spreading of microbial contamination (Ali *et al.*, 2010).

The commonest way for prolonging the shelf life of meat is by cooling. Two methods for preserving meat through low temperatures, namely chilling and freezing, can be applied. For chilling, meat is refrigerated at a temperature of 0°C to 4°C, and at -18°C for freezing. As the temperature becomes colder, the enzyme action, and the growth and development of bacteria become (Johanna, 2005). Both chilled and frozen imported types of meat are considered a significant source of bacteriological public health hazards and need special control attention (Hassanien *et al.*, 2020).

Many studies have investigated the effect of chilled storage duration which pretended to be the critical factor in terms of maintaining meat quality and preventing spoilage for export pur-

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poses and other activities (Leygonie et al., 2012). Egypt imports different types of meat to fill the gaps in the requirements of animal protein (USDA, 2016). Whereas the maintenance of hygiene and sanitation is always questionable.

Total bacterial counts (TBC) and Enterobacteriaceae counts (EC) are good indicators of meat quality and its spoilage. Moreover, microbial quality of chilled meat obtained from markets can vary greatly, for these reasons, food products are being examined intensively for microbial contamination, especially during export/ import or marketing across boundaries. Therefore, this study was conducted to evaluate the bacteriological quality of imported chilled meat traded in Port-Said markets via determination of TBC, Enterobacteriaceae count, and detection of Escherichia coli, S. aureus, and Salmonella.

MATERIALS AND METHODS

Collection of samples

A total of 64 random samples (250 g weight of each) of imported chilled meats represented by 28 imported chilled beef samples from lots arrived at Port-said port (un-marketed) and 36 imported chilled beef samples collected from retailed markets at Port-said governorate (marketed), the recommended sampling sites were neck, brisket, rump, and flank cuts. All accrued samples have been subjected to bacteriological examination at Port-said analytical lab, Animal Health Research Institute, Egypt.

Preparation of samples

All samples were prepared according to the technique recommended by APHA (2001). Each sample was separately placed into clean sterile plastic bags and transferred in an insulted icebox to the laboratory without delay under complete aseptic conditions. Flaming the surface of a 250 g sample from each site and removing it then cutting an area of 25 g from the area under the removed surface (70% ethanol flamed, between uses). The 25 g from each sample were transferred under aseptic condition to a sterile polyethylene bag containing 225 mL of 0.1% sterile buffered peptone water. The content of the bag was then homogenized using a stomacher (Lab. Blender 400, Seward Lab, London) to have a dilution of 10-1 then One ml of each diluent was used in the preparation of serial dilution and the rest was used as a pre-enrichment medium for isolation of Salmonella. The serial dilution was used in the determination of total aerobic counts (APHA, 2015), Enterobacteriaceae counts (ISO, 2017), total Escherichia coli (FDA, 2020) Staphylococcus aureus (ISO, 1999), and Salmonella (ISO, 2002a).

Statistical analysis

Data analysis was performed by using SPSS statistical software program (SPSS for Windows version 16, Spss Inc., USA). Data were expressed as mean ± standard error (SE). Two-way analysis of variance (ANOVA) with Duncan post-hoc multiple comparisons test. Any significant differences (P<0.05) were analyzed by the multiple comparisons' procedure of LSD (least significant difference), using a level of significance of alpha = 0.05.

RESULTS AND DISCUSSION

Meat is considered a rich nutrient matrix that provides a suitable environment for the proliferation of meat spoilage microorganisms and common foodborne pathogens.

From the results of this study, the total bacterial count (cfu/g) of un-marketed and marketed imported chilled meat examined at Port-Said lab in different meat cuts recorded an average from 10.73x10⁴ to 2.5x10⁶, respectively. When it was discussed, as recorded in Table 1, TBC showed a highly significant difference among different meat cuts; neck, brisket, flank, and rump in un-marketed imported chilled mat which recorded mean values of 3.6x10³, 2.6x10³, 3.8x10⁵ and 4.3x10⁴ respectively, these differences may be attributed to the location of each meat cut and handling practices, certain areas of the carcass are more likely to be contaminated or to remain contaminated than other parts. For these reasons, microorganisms are not uniformly distributed over the carcass (NAS, 1985). Also, high total bacterial counts (cfu/g) of marketed imported chilled meat at Port-Said markets in different meat cuts; neck, brisket, flank, and rump were noticed as 3.6x10⁵, 3.1x10⁴, 4x10⁶, and 5.5x10⁶ respectively. Hassanien et al. (2020) disagreed with the obtained results in aerobic plate count while Mansour and Basha (2009) nearly agree with the present study which was 3.4x10⁴ in frozen cuts also Saleh et al. (2013) revealed similar results when recorded 1.4x10⁶, 6.5x10⁵ in the fore quarter and 9.6x10⁵, 1.2x10⁶ in the hind quarter.

The prevalence of TBC in Table 3, revealed that 42.85% and 52.77% of un-marketed and marketed imported chilled meat respectively, contained microorganisms. However, the counts were

	Total bacte	erial count	T 1 1 4 4 4 4	Enterobacter	Independent t-test	
Sites	Mean	±S.E.	Independent t-test	Mean		
	Un-marketed (n=28)	Marketed (n=36)	p-value	Marketed (n=36)	Un-marketed (n=28)	p-value
Neck	$3.6 x 10^3 \pm 1.1 \ x 10^2$	4.5x10 ⁵ ± 1.1 x10 ⁴	< 0.001***	2.6x10±1.1 x10	3.0x10±1.1 x10	0.394 ns
Brisket	$2.6 x 10^3 \pm 2.1 \ x 10^2$	$5.1 x 10^4 {\pm}~2.1~x 10^2$	0.967 ns	3.1x10 ³ ±2.1 x10 ²	3.1x10 ² ±2.1 x10	0.003**
Flank	$3.8 x 10^{5} \pm 1.3 x 10^{3}$	$4x10^{6}\pm 1.3 x10^{4}$	0.018*	4.3x10 ³ ±1.3 x10 ³	3.2x10 ² ±1.3 x10 ²	0.007**
Rump	$4.3x10^{4}\pm2.0\ x10^{3}$	$5.5 x 10^6 \pm 2.0 \ x 10^3$	< 0.001***	5.5x10 ³ ±2 x10 ³	3.5x10 ³ ±2 x10	<0.001***
Total	$10.73 x 10^4 {\pm}~6.6 x 10^4$	$2.5 x 10^6 \pm 10.8 x 10^5$	< 0.001***	$3.29x10^3 \pm 4.99x10^2$	$1.04 x 10^{\scriptscriptstyle 3} \pm 3.4 x 10^{\scriptscriptstyle 2}$	< 0.001***
ANOVA	<0.001***	<0.001***		<0.001***	<0.001***	
			Two-way ANO	VA		
Corrected Mo	del	< 0.001***			<0.001***	
Group		< 0.001***			< 0.001***	
Sites		0.002**			< 0.001***	
Group x Sites		0.004**			0.592 ns	

Table 1. Bacteriological quality results of imported chilled meat for different carcass sites

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	Total E. c	coli count	Independent t-test p-value	Staphylococcu	Independent t-test	
Sites	Mean	±S.E.		Mean		
	Un-marketed (n=28)	Marketed (n=36)		Un-marketed (n=28)	Marketed (n=36)	p-value
Neck	0.7x10±1.2 x10	< 10± 0 x10	0.420 ns	0.6x10±1 x 10	0.6x10 ² ±0.1 x10	< 0.001***
Brisket	2.1x10 ² ±2.1 x10	3.1x10 ³ ±2.1 x10 ³	< 0.001***	$<10\pm0$ x 10	0.3x10±0.1 x10	0.045 *
Flank	$1.3x10^2 \pm 1.3 x10^2$	$4x10^{2}\pm1.3 x10^{2}$	0.793 ns	0.4x10±1.3 x 10	$< 10 \pm 0 \text{ x} 10$	0.940 ns
Rump	$1.5 x 10^2 \pm 0.5 x 10^2$	$5.5x10^3 \pm 2 \ x10^3$	< 0.001***	$0.5 x 10 \pm 2 \ x 10^2$	$2.1x10^2 \pm 0.2 \ x10^2$	>0.05 ns
Total	$1.40 x 10^2 \!\!\pm 1.70 x 10^1$	$2.25 x 10^3 {\pm}~4.52 x 10^2$	< 0.001***	$0.63 x 10 {\pm}~1.35 x 10$	$7.08 x 10 \pm 1.93 x 10 \\$	0.008**
ANOVA	<0.001***	<0.001***		0.999 ns	<0.001***	
			Two-way ANOVA			
Cor. Model		<0.001***			< 0.001***	
Group		<0.001***			0.002**	
Sites		< 0.001***			0.004**	
Group x Sites		< 0.001***			0.001***	

Table 3. The prevalence of total bacterial count and Enterobacteriaceae in imported chilled meat for different carcass sites

		Total bacterial counts		Chi aguana	Enterobacteriaceae		Chiarman
Variable		Frequency (n, %)		Chi-square	Frequency (n, %)		Chi-square
		Un-marketed (n=28)	Marketed (n=36)	p-value	Un-marketed (n=28)	Marketed (n=36)	p-value
	Positive	12 (42.86%)	19 (52.78%)	0.803 ns	5 (17.86%)	13 (36.11%)	<0.001***
Record	Negative	16 (57.14%)	17 (47.22%)	0.803 ns	23 (82.14%)	23 (63.89%)	<0.001
	MPL	106	106		102	102	
Acceptability	Accepted	25 (89.29%)	29 (80.56%)	<0.001***	27 (96.43%)	31 (86.11%)	<0.001***
Acceptability	Unaccepted	3 (10.71%)	7 (19.44%)	<0.001****	1 (3.57%)	5 (13.89%)	<0.001

*, **, ***: Significant at p<0.05, <0.01, <0.001; NS: Nonsignificant at p>0.05. MPL = Maximum permissible limit according to ESS (3602/2013) for chilled meat.

considered satisfactory as these results were lower than those suggested by EEC (2005). Only 3 samples were unaccepted, based on the limit of TBC established by the (ESS) which is 10⁶ cfu/g in chilled meat, from the un-marketed samples out of 28 which represents 10.7%, while there were 7 unaccepted samples out of 36 in marketed samples by 19.4%, the lowest results were recorded by Saleh *et al.* (2013), 4% and the highest were recorded by Zafar *et al.*, (2016) in Karachi.

The high counts of total aerobic bacteria may be due to the homemade dressing of corpse hides at the hands of the abattoir workers. Generally, hygiene conditions are poor when foods are produced in non-industrial establishments, substantially because the necessary structure for technologically acceptable processes isn't available. Corruption or reduce keeping the life of stupefied meat can be generally attributed to the presence of a veritably large number of bacteria, these were substantially linked as members of psychotropic bacteria and certain other microorganisms able of growing at 0°C.

For *Enterobacteriaceae*, it is evident from the results given in Table 1, which showed its mean values in un-marketed imported chilled meat samples were $3.0 \times 10 \pm 1.1 \times 10$, $3.1 \times 102 \pm 2.1 \times 10$, $3.2 \times 10^2 \pm 1.3 \times 10^2$ and $3.5 \times 102 \pm 2 \times 10$ (cfu/g) for neck, brisket, flank, and rump, respectively. Higher counts were recorded for total *Enterobacteriaceae* counts of marketed samples, where its mean values were $2.6 \times 10 \pm 1.1 \times 10$, $3.1 \times 10^3 \pm 2.1 \times 10^2$, $4.3 \times 10^3 \pm 1.3 \times 10^3$, $5.5 \times 10^3 \pm 2 \times 10^3$ (cfu/g) in the neck, brisket, flank, and rump respectively. Nearly similar results were recorded by Hassan and Soultan (2004); Stagnitta *et al.* (2006); Gwida *et al.* (2014) and Shaltout *et al.* (2016). While Zerabruk *et al.* (2019) found that *Enterobacteriaceae* is the dominant microflora of meat. Most of the *Enterobacteriaceae* present in meats come from fecal contaminations. Elevated numbers of *Enterobacteriaceae* can be used as an

indicator for poor hygienic conditions during handling or inadequate storing of chilled meat and the bacterial contamination quality of chilled raw meats.

Table 2 reveals the *E. coli* counts in un-marketed and marketed imported chilled meat. Where the mean values were 0.7x10 \pm 1.2x10, 2.1x10² \pm 2.1x10, 1.3x10² \pm 1.3x10² and 1.5x10² \pm 0.5x10² in neck, brisket, flank, rump respectively in un-marketed samples. Higher *E. coli* count in marketed samples with mean values of <10 \pm 0 x10, 3.1x10³ \pm 2.1x10³, 4x10² \pm 1.3x10², and 5.5x103 \pm 2x103 in the neck, brisket, flank, and rump respectively.

Table 2 displays the Staph. aureus counts in the un-marketed imported chilled meat with mean values of 0.6x10 ±1x10, <10 \pm 0x10, 0.4x10±1.3x10 and 0.5x10±2x10² in neck, brisket, flank and rump correspondingly. Those consequences have disagreed with the ones obtained by Abd El-Hady (2015), Abdaslam et al., (2014), El Jakeeet al. (2014), and Tarabees et al. (2015) who isolated S. aureus from raw meat and meat products with higher incidence. The same table reveals the Staph aureus counts results from marketed chilled meat with a means of 0.6x10²±0.1x10, $0.3x10\pm0.1x10$, <10±0x10, and 2.1x10²±0.2x10² in the neck, brisket, flank, and rump respectively, which are nearly similar to that obtained by Morshedy et al. (2013) who mentioned that the mean value of S. aureus was 4.3×102cfu/g. and to that obtained by Shaltoot et al. (2016) who found that the incidence of Staph. aureus was low with a mean value of 2.2 ± 0.07 log10, Attalla and Kassem (2011) recorded that the mean value of staph aureus count was 3.94 ±0.16 log10, and Azage and Kibret (2017) who found that the mean value of Staph. aureus was 3.88 log10 cfu/g for the examined samples. While higher results were obtained by El Kewaiky and Al Said (2015) who found that the mean value of S. aureus was 5.26±4.7 log10 in examined fresh samples and 4.4±3.88 log10 in frozen samples

Table 3, showed that only 1 sample was unaccepted for *Enterobacteriaceae* count, based on the limit of *Enterobacteriaceae* counts established by the ES (2005) which is 10² cfu/g chilled meat, from un-marketed samples out of 28 which represents 3.57%, while there were 5 unaccepted samples out of 36 in marketed chilled meat samples by 13.89%.

Considering the pathogenic parcels of *Enterobacteriaceae* and their high frequency in meat, strict observance of hygiene programs and systematical monitoring of *Enterobacteriaceae* at all stages of food products plays a pivotal part in icing food safety and controlling the transmission of pathogenic foodborne bacteria to humans.

Table 4 showed the acceptability of the examined chilled meat samples based on their *E. coli* count (n = 28, 36), where the percentage of the unaccepted samples were 7.14% and 11.11% in 28 un-marketed and 36 marketed imported *E. coli* chilled meat samples, respectively.

The same findings of E. coli incidence have been mentioned via Mansour and Basha (2009) who remoted E. coli from 8 % of the tested frozen meat and Ukut et al. (2010) who determined E. coli in 11.1% of samples that were gathered in Nigeria from two foremost markets. Meanwhile, a decreased result has been pronounced in Egypt through Elnawawi et al. (2012) who isolated E. coli O158 and E. coli O86 from samples of imported frozen meat, with a percent of 2.86% and 1.42%. In addition, different E. coli species were isolated from 5.71% of imported frozen meat. Additionally, Ahmed and Shimamoto (2013) in Egypt investigated the prevalence of some foodborne pathogens in meat samples gathered from slaughterhouses, butchers, street vendors, and retail markets, E. coli O157:H7 have been detected in 3.4%. The highest levels of E. coli were reported by Martinez et al. (2015) who found that E. coli is present in 97% of beef carcasses. High percentages of Escherichia coli are often used as hygiene indicators of foods of animal origin. Its presence in chilled meat may give a better indication than coliforms of inadequate treatment or post-process contamination from the environment and may help to indicate the extent of fecal contamination.

The presence of staphylococci is normally associated with human pores and skin and apparel, it additionally may be a result of unhygienic dealing with and processing using unclean knives, slicing boards, and garage ladders added to the terrible hygienic reputation of meals handlers in butchers' stores (Gebeyehu *et al.*, 2013). *Staphylococcus aureus* is one of the most causative pathogens to developed foodborne illnesses causing an estimated quarter-million cases every year in the US. *S. aureus* is one of the most important food poisoning microorganisms (Naomi and Avraham, 2000) these pathogens are of major concern to the meat industries (Hannan *et al.*, 2008; Morshdy *et al.*, 2018).

Results given in Table 4 showed that the prevalence of *S. aureus* in the un-marketed samples was 3.57% and 8.33% for marketed samples. And for all the examined samples, there were 4 unaccepted samples out of 64 chilled meats (un-marketed and marketed). A nearly similar result was obtained by Iraha *et al.* (2011) who recorded the incidence of *Staph. aureus* was 1.3%, while a slightly higher incidence was obtained by Tassew *et al.*, (2010) who found that the incidence of *S. aureus* was 12.1%.

On the other hand, a high incidence was achieved by Maarouf and Nassif (2008); Lamada *et al.* (2012) and Abdel Salam *et al.*, (2014) who found that the incidence of *S. aureus* was 35.4% in the examined samples, Salek, (2000) who found that in 28 out of 61 samples the *S. aureus* incidence was 45.9%, also Gwida *et al.*, (2014) who recorded incidence of *S. aureus* 48% and Tang *et al.*, (2017) recorded 68% in Denmark. This difference in the prevalence of S aureus may be reflected in the sample sizes, sample types, hygienic measures, collection time, geographic locations, sanitary conditions of handlers, and the detection procedures used (Ge *et al.*, 2017). According to the Egyptian standard specification ESS (3602/ 2013) for chilled meat, it has been proposed

Table 4. The prevalence of E. coli and Staphylococcus aureus in imported chilled meat for different carcass sites

	Variable	<i>E. coli</i> Frequency (n, %)		Chi-square	Staphylococcus aureus Frequency (n, %)		Chi-square
		Un-marketed (n=28)	Marketed (n=36)	p-value	Un-marketed (n=28)	Marketed (n=36)	p-value
Record	Positive	2 (7.14%)	4 (11.11%)	<0.001***	1 (3.57%)	3 (8.33%)	<0.001***
	Negative	26 (92.86%)	32 (88.89%)		27 (96.43%)	33 (91.67%)	
	MPL	Free	Free		Free	Free	
Acceptability	Accepted	26 (92.86%)	32 (88.89%)	<0.001***	27 (96.43%)	33 (91.67%)	<0.001***
	Unaccepted	2 (7.14%)	4 (11.11%)		1 (3.57%)	3 (8.33%)	<0.001****

*, **, ***: Significant at p<0.05, <0.01, <0.001; NS: Nonsignificant at p>0.05. MPL = Maximum permissible limit according to ESS (3602/2013) for chilled meat.

Table 5. The prevalence of Salmonella sp. in imported chilled meat for different carcass sites

		Salmone	Salmonella sp.				
	Variable	Frequenc	Frequency (n, %)				
		Un-marketed (n=28)	Marketed (n=36)	p-value			
	Positive	0 (0.00%)	2 (5.56%)	<0.001***			
Record	Negative	28 (100.00%)	34 (94.44%)				
	MPL	free	free				
	Accepted	28 (100.00%)	34 (94.44%)				
Acceptability	Unaccepted	0 (0.00%)	2 (5.56%)	< 0.001***			
	Unaccepted	0 (0.00%)	2 (5.56%)				

*Significant at p<0.05, ** significant at <0.01, *** significant at <0.001; NS, non-significant at p >0.05. MPL = Maximum permissible limit according to ESS (3602/2013) for chilled meat.

that chilled meat must be free from S. aureus.

The existing not succeed to detect *Salmonella* species from all examined un-marketed samples (Table 5), These results were agreed with those recorded by Elnawawi *et al.* (2012); Datta *et al.* (2012), Saleh *et al.* (2013) and Abdel-Raouf *et al.*, (2014) also Abuelnaga *et al.*, (2021) which failed to isolate *Salmonella* species from any of examined beef samples. While, disagreed with those given by Abdaslam *et al.* (2014); Maarouf and Nassif-Marionette (2008) and Ramadan (2015) who isolated *Salmonella* from beef and meat products.

On the other hand, *Salmonella* was detected in the marketed samples in 2 over 36 samples as 5.5% and the 2 samples were unaccepted (table 5), Nearly the same results were reported by Rhoades *et al.* (2009) for fresh meat samples detected *Salmonella* by in average 3.8% (0.0–7.5%) on raw beef samples. Higher results were detected by Ukut *et al.* (2010) who detected *Salmonella* spp. in 11.1% of the fresh meat in Nigeria. According to ESS (3602/2013) for chilled meat, all meat samples must be free from *E. coli* and *Salmonella* spp.

This study showed that there is a highly significant difference (p < 0.001) between un-marketed and marketed chilled meat (marketed meat contained higher microbial content) and proved that raw retail chilled meats and meat shops are potential vehicles for transmitting meat-borne diseases, the findings of this study stress the need for increased consumer meat-safety education efforts.

CONCLUSION

In the light of the previously achieved results, it was concluded the poor bacteriological quality of some imported chilled meat that is marketed in Port-Said retailed markets which related to unhygienic transportation methods until reaches the retailed markets, refrigeration, and unsanitary handling during marketing and selling process of meat, which limits the opportunities of meat storage and commercial life and causes many foodborne diseases to human. Therefore, further studies should be carried out to establish the control measures that should be adapted to improve the bacteriological quality of imported chilled meat traded in markets to ensure meat safety.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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