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Bacteriological quality guides in local and imported beef and their relation to public health

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ABSTRACT

Contamination of meat products are thought to be the most significant key sources of serious diseases, especially foodborne diseases that spreading all over the world. A total of 100 random samples of chilled local and frozen imported beef (50 of each) were collected from local supermarkets and retail shops from Cairo governorate for evaluation of their quality from bacteriological point of view. Aerobic plate count (CFU/g)in the local samples were between 1.1×10^6 and 4.4×10^7 with an average of $4.1\times10^7\pm 0.02\times10^6$ while , in frozen imported were ranged between 2.6×10^7 to 5.3×10^8 with an average of $2.8\times10^7\pm 0.03\times10^7$. Moreover, Coliform count (CFU/g) in local samples were ranged from 1×10^3 to 1.2×10^4 with average of $4.2x10^3 \pm 0.03\times10^3$, while in imported samples were between 6×10^2 and 11.0×10^3 with average of $7.1x10^3 \pm 0.02\times10^3$. *Escherichia coli* were detected in 4% of chilled samples and 2% of frozen samples. *Salmonella* spp. has been detected in 4% of local samples while all frozen samples were free. From the overall results, we can conclude that both chilled local and frozen imported meat are considered as a significant source of bacteriological public health hazard and need a special control attention.

1. INTRODUCTION

Meat is considered as one from the most nutrient-dense food that provides ideal conditions for microbes to grow and defines its perishable nature (Saucier, 2016). The high level of moisture of meat is corresponding to the water activity of roughly 99%, which is appropriate for the growth of different types of microorganisms (Rao et al., 2009). In contrast, meat products are thought to be the most significant key sources of diseases, especially foodborne diseases that outbreaks resulting from food poisoning, all over the world. Therefore, bacterial food poisoning cases, particularly that are caused by Salmonella spp., which are the main source of the contamination of meat products worldwide (Reham, 2004). Meat in general could be considered as poor hygienic quality or unfit for human consumption when the APC exceeds 106CFU\g (Alberle et al., 2001) . Many factors may be contributed as sources of contamination of carcasses along the chain of slaughter, including the animal's skin and dung, equipment and a lack of personal hygiene. (Boukhors et al., 2012). Although muscles of healthy animals do not contain microorganisms, meat tissues get contaminated during the various stages of slaughter and transportation. The risk of contamination happens from the point of entry of animals into the slaughters up to the time of meat consumption. In this regard, the abattoir environments and slaughter processes play leading roles in the spreading of microbial contamination (Ali et al., 2010). A large-scale study about the prevalence of some foodborne pathogens in meat samples collected from street vendors, butchers, retail markets and slaughterhouses in Egypt, Salmonella enterica and E. coli were detected in 69 (4.3%), 54 (3.4%) and 27 (1.7%) samples, respectively (Ahmed et. al., 2013). It was reported that many studies have investigated the effect of frozen storage duration which appears to be the critical factor in terms of maintaining meat quality and preventing spoilage for export purposes and activities. (Leygonie et al., 2012). *E. coli* is generally non-virulent, but some strains have adopted pathogenic or toxigenic virulence factors that make them serious for human and animals. and cross contamination with bacteria either in homes or in food service establishments which is thought to be a major factor for sporadic and epidemic foodborne illness. (Donald et al., 2001). Safe food could be defined as food that is hazards - free products, whether chronic or acute hazards, that may make food hazardous to the health of the consumers. (WHO and FAO, 2003). The most of Salmonella spp. and E. coli were found to be the cause of serious foodborne diseases, they are also involved in spoilage of foods. Furthermore, they cause a great threat to human health as well as in country's economy. The sanitary conditions of the slaughterhouses, butcher shops, handling of meat, environmental condition and improper packing and selling of meat play important roles in the level of contamination. Contaminated raw meat is the main source of foodborne illnesses (Bhandare et al., 2017). Consequently, the current study was planned out to discuss briefly the incidence of Bacterial contamination in local and imported meat samples collected from Egyptian markets.

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2. MATERIAL AND METHODS

2.1. Sampling:
A total of 100 random chilled and frozen beef samples (50 0f each) were collected from different retail shops in Cairo. The imported meat samples were collected from raw frozen imported meat stored at a storage temperature -18°C to -20°C which were collected during their claimed shelf life time, while the local fresh meat samples were collected from raw chilled meat stored at 4°C to 8°C , collected during the claimed shelf life time .Then all samples were transferred directly to the laboratory, in an ice-box under complete aseptic conditions without any delay. Then they were subjected to following examinations to evaluate bacteriological quality.

2.2. Preparation of samples (ISO, 2017):
Under complete aseptic condition, twenty-five grams of the examined minced meat samples were transferred to aseptic stomacher bag and 225 ml of 0.1% sterile peptone water were aseptically added to the content of bag After thawing to samples in the refrigerator overnight. Each sample was homogenized, in the stomacher (Biomereuxsa – France – NO.42489367) at 2000 r. p. m for 1-2 minutes to provide a homogenate from which tenth – fold serial dilutions were prepared. This is done by adding 1ml from homogenate to 9ml of 0.1% sterile peptone water tube then take 1 ml from this tube by sterile pipette to another sterile test tube containing 9ml of sterile peptone water 0.1% and mix well to make the next dilution and so on. The prepared samples were subjected to the following examinations.

2.3. Determination of Total coliforms count and Aerobic plate count "APC"
Total coliforms count was carried out according to ISO, (2004) while Aerobic plate count was carried out according to ISO, (2013)

2.4. Isolation, identification and characterization of E. coli (ISO 2001)
Isolation and serological identification of E. coli was performed (ISO 2001), then morphological examination by Gram's Stain (Cruickshank et al., 1975) and their Motility (Mac Faddin, 2000). Biochemical characterization of isolated bacteria using Indole test (Mac Faddin, 2000), Methyl Red test (Mac Faddin, 2000), Voges Proskaur test (Cheesbrough, 1985), Citrate utilization test (Mac Faddin, 2000), Hydrogen supplied production test (Mac Faddin, 2000),Gelatin hydrolysis test(Mac Faddin, 2000), Urease test (Mac Faddin, 2000), Eijkman test (Mac Faddin, 2000), Nitrate reduction test (Mac Faddin, 2000) and Sugars fermentation (Mac Faddin, 2000).

2.5. Isolation, identification and characterization of Salmonella (ISO 2017):
Isolation was done on Pre- enrichment in non-selective liquid media (peptone water (0.1%), Enrichment (on Rappaport Vassilidis broth) and Selective plating (on previously prepared Xylose Lysine Desoxycholate (X.L.D) agar). The isolates were morphologically examined by Gram's Stain (Cruickshank et al., 1975) and their Motility (Mac Faddin, 2000). Biochemical characterization of isolated bacteria using Indole test (Mac Faddin, 2000), Methyl Red test (Mac Faddin, 2000), Voges Proskaur test (Cheesbrough, 1985), Citrate utilization test (Mac Faddin, 2000), and Sugars fermentation (Mac Faddin, 2000). Serological identification of Salmonella was carried out according to Kauffman – White scheme for the determination of Somatic (O) and flagella (H) antigens using Salmonella antiserum (DENKA SEIKEN Co., Japan)

3. RESULTS

Results in tables (1) showed the Bacteriological evaluation of chilled and frozen samples and revealed that Aerobic plate count (CFU/g)in the examined chilled local samples were ranged from 1.1×106 and 4.4 ×107 with an average of 4.1×107± 0.02×106 , while in frozen imported samples were ranged between 2.7×107 to 5.3 ×108 with an average of 2.9×107± 0.02 ×107 .
Table (2) revealed that all examined samples, either chilled or frozen are unaccepted for human consumption due to bad hygienic indication comes from APC according to Egyptian standard specification ES (3602/2013)for fresh meat and ES (1522/ 2005) for frozen meat.
As shown in table (3) Coliform count (CFU/g) were ranged from 1×10^3 to 12.4×10^3 with average of $4.2x10^3\pm 0.03\times10^3$ and 6×10^2 to 11.0×10^3 with average of $7.1x10^3 \pm 0.02\times10^3$ in chilled local and frozen imported samples.

Table 1 statistical analytical results of Aerobic plate count (CFU/g) in the examined beef samples (n = 50 for each).

Meat samples	Min.	Max.	Mean ± S.E.*
Chilled local	1.1×10^6	4.4×10^7	$4.1\times10^7\pm 0.02\times10^7$
Frozen imported	2.7×10^7	5.3×10^8	$2.9\times10^7\pm 0.02\times10^7$

*S. E.= Standard error of mean. N.B: No Significant difference (P > 0.05).

Table 2 Acceptability of the examined beef samples based on their APC/g (n = 50).

Meat samples	+ve samples		MPL[1]	accepted samples		Unaccepted samples	
	No.	%		No.	%	No.	%
Chilled local	50	100%	10^6	-	-	50	100
Frozen imported	50	100%	10^6	-	-	50	100

MPL[1] = Maximum permissible limit according to EOS (3602/2013)for fresh meat and (1522/ 2005) for frozen meat.

Table 3 Statistical analytical results of Coliform count (CFU/g) in the examined beef samples (n = 50).

Meat samples	Min.	Max.	Mean ± S.E.*
Chilled local	1×10^3	12.4×10^3	$4.2x10^3 \pm 0.03\times10^3$
Frozen imported	6×10^2	11.0×10^3	$7.1x10^3 \pm 0.02\times10^3$

S. E.= Standard error of mean. N.B: No Significant difference ((P>0.05).*

According to table (4), 40 % of chilled local samples were not accepted for human consumption because they were higher than the permissible limit declared by ES (3602/2013) for chilled meat and ES (1522/2005) for frozen meat., while 30% of frozen samples were unfit for public consumption in terms of Coliform count
Results in table (5) illustrated that the incidence of *E. coli* were 4% in chilled samples and 2% of frozen ones
From table (6) showed the serotyping of *E. coli* isolated from the examined fresh and frozen meat, *E.coli* O55 : H7 has been detected only in 2% of fresh meat samples and *E.coli O125* : *H18* was detected in 2% of chilled local meat samples , while *E.coli O114 : H21* were detected in 2 % of frozen meat samples.
For *Salmonellae* examination, the film revealed Gram – coccobacilli to medium size rods, stained evenly and non-sporulated with rounded end. Salmonella showed positive reaction (circular growth around the line of stabbing). They were Negative (yellow color) for indole test and Voges

Proskauer and positive (red color) for methyl red, Citrate utilization and Sugar fermentation.

As shown in table (7) *Salmonellae* were detected in 4% in chilled samples while failed to be detected in frozen samples.

According to ES (3602/2013) for fresh meat and ES (1522/ 2005) for frozen meat, that all meat samples must be free from *E. coli* and *Salmonella spp*

Table 4 Acceptability of the beef samples based on their Coliform count (n = 50 each).

meat samples	+ve samples No.	%	MPL[1]	accepted samples No.	%	Unaccepted samples No.	%
Chilled local	20	40	free	30	60	20	40
Frozen imported	15	30	10^2	35	70	15	30

MPL[1] = Maximum permissible limit according to EOS (3602/2013)for fresh meat and (1522/ 2005) for frozen meat.

Table 5 Incidence and acceptability of *E. coli* isolated from the examined beef samples (n= 50 for each)

Meat samples	+ve samples No.	%	MPL[1]	accepted samples No.	%	Unaccepted samples No.	%
Chilled local	2	4	free	48	96	2	4
Frozen imported	1	2	free	49	98	1	2

MPL[1] = Maximum permissible limit according to EOS (3602/2013)for fresh meat and (1522/ 2005) for frozen meat.

Table 6: Serotyping of *E. coli* isolated from the examined beef samples (n= 50 for each).

Meat samples *E. coli* Strains	Chilled local No.	%	Frozen imported No.	%	Strain characteristics
O55 : H7	1	2	-	-	EPEC
O114 : H21	-	-	1	2	EPEC
O125 : H18	1	2	-	-	ETEC
Total	2	4	1	2	

EPEC = Enter pathogenic *E. coli.* ETEC = Enter toxigenic *E. coli*

Table 7 Incidence and acceptability of *Salmonella* isolated from the beef samples (n= 50).

Meat samples	+ve samples No.	%	MPL[1]	accepted samples No.	%	Unaccepted samples No.	%
Chilled local	2	4	free	48	96	2	4
Frozen imported	-	-	free	50	100	-	-

MPL[1] = Maximum permissible limit according to EOS (3602/2013)for fresh meat and (1522/ 2005) for frozen meat.

4. DISCUSSION

Food is an excellent vehicle by which many pathogenic microorganisms can reach an appropriate host (Newell et. al., 2010) . The results of Aerobic plate count in the examined chilled local meat samples were between 1.1×10^6 CFU/g. and 4.4×10^7 CFU/g. with an average of $4.1\times10^7\pm 0.02\times10^7$CFU/g. while the examined frozen samples were ranged between 2.7×10^7 CFU/g. to maximum 5.3×10^8 CFU/g. with an average of $2.9\times10^7\pm 0.02\times10^7$ CFU/g.. This clearly indicates to how the fresh samples could be contaminated during handling, storage and transportation. The results revealed that all examined samples, either local chilled or frozen are unaccepted for human consumption due to bad hygienic indication comes from APC according to Egyptian standard specification ES (3602/2013)for local chilled meat and ES (1522/ 2005) for frozen meat. Sujiwo et al. (2019) has got close results at day 12 of cold storage at 4 °C. It was 6.87 CFU/g. While got higher results, 8.61 CFU/g at day 15 of storage in the same conditions.

Ercolini et. al. (2010) got almost similar results for total aerobic bacterial count on day 22 of storage , as reported 7.13 CFU/g on day 22 of storage. However, higher results were obtained by McCain (2015) who determined the influence of market type and sampling time on Aerobic bacteria counts which ranged from 10.5 to 11.6 CFU/g in beef in Vietnam.

On the other hand, lower total aerobic plate count was 4.3×10^4 CFU/g in frozen beef cuts which recorded by Mansour and Basha (2009) and also lower results was reported by Hassanin et al. (2017) at Basatin abattoir in summer when the results of the total aerobic bacterial count in the following region A1, A2, A3, B1, B2, B3 in hall 1 were $3.8\times10^4 \pm 3.4x\ 10$; $3.5\ x10^4 \pm 2.1\ x10^3$; $4.7\ x10^4 \pm 3.1\ x10^3$; $3.3\ x10^4 \pm 1.1\ x10^3$; $3.3\ x10^4 \pm 4.4\ x10^3$; $4.4\ x10^4 \pm 4.3\ x10^3$; respectively.

The results of coliforms count for fresh samples were ranged from 1×10^3 CFU/g. to 1.2×10^4 CFU/g. with average of $4.2x10^3 \pm 0.03\times10^3$ CFU/g. , while frozen samples ranged between 6×10^2 CFU/g. and 1.1×10^4 CFU/g. with average of $7.1x10^3 \pm 0.02\times10^3$ CFU/g.

Based on total coliform count, 40 % of fresh samples were not accepted for human consumption because they were higher than the maximum permissible limit declared by ES (3602/2013) for local chilled meat and ES (1522/ 2005) for frozen meat., while 30% of frozen samples were unfit for public consumption for the same reason .These results were consistent with those obtained by Ukut et al. (2010) who collected from two major markets in Nigeria and revealed that fresh meat are commonly contaminated with pathogenic bacteria. The total coliform count were between $1.1\ x\ 10^3$ - $3.7\ x\ 10^3$ CFU/g while the total coliform count of fresh meat from the other market were between $1.2\ x\ 10^3$ - $3.4\ x\ l0^3$CFU/g. Scanga et al. (2000) obtained lower results when they made a survey for the microbiological status of beef trimmings, The final products samples were evaluated for total coliform (TCC), 1.3± 0.3 log CFU/g., 1.5± 0.4 log CFU/g.

While, McCain (2015) reported too much higher coliform counts which ranged from 7.2 to 11.4 CFU/g of beef in Vietnam. Results concluded by Donald et al. (2001) and Datta et al. (2012) revealed that *E. coli* is generally non-virulent but some strains have adopted pathogenic or toxigenic virulence factors that make them serious for human and animals, also cross contamination with bacteria either in homes or in the markets is thought to be a major factor for sporadic and epidemic foodborne illness. However, Caprioli et al. (2005) reported that, *E. coli* is the major foodborne pathogen that has gained an enlarged interest of researches in the last few years. The results revealed that the Incidence of *E. coli* isolated from the examined fresh and frozen meat which detected in 4% and 2% of fresh and frozen samples respectively. ES (3602/2013) for fresh meat and (1522/2005) for frozen meat, that all meat samples must be free from *E.coli* to be accepted for human consumption. The results showed that the Serotyping of *E. coli* isolated from the examined local chilled and frozen meat, *E.coli O55:H7* has been detected only in 2% of fresh meat samples and *E.coli O125 : H18* was detected in 2% of fresh meat samples , while *E.coli O114 : H21* were detected in 2 % of frozen meat samples. Both *E. coli O55: H7* and *O114: H21* are characterized as EPEC strains while *E.coli O125: H18* is characterized as ETEC strain

Almost the same results of *E. coli* incidence were reported by Ahmed and Shimamoto (2013) when conducted a

large-scale study to investigate the prevalence of some foodborne pathogens in meat samples collected from street vendors, butchers, retail markets and slaughterhouses in Egypt. *E. coli O157:H7* were detected in 3.4% of samples and found that *E. coli O157:H7* was higher in dairy products than in meat products. However higher results were reported by Ukut et al. (2010) as they found *E. coli* in 11.1% of samples which were collected in Nigeria from two major markets. Also, Mansour and Basha, (2009) isolate *E.coli* from 8 % of the examined frozen meat samples, *E.coli* strains were serotyped as O55, O111, O114 and O119. More tragic results were found by Martínez et al., (2015) who reported that *E. coli* presence in 97% of beef carcasses. *E.coli* mean counts were 3.2 ± 0.7 Log CFU/300 cm^2 on beef carcasses. On the other hand, a lower result has been reported in Egypt by Elnawawi et al. (2012) who isolated *E. coli O158* and *E. coli O86* from samples of imported frozen meat. with percentage 2.86% and 1.42%. In addition, other *E. coli* species were isolated from 5.71 % of imported frozen meat. It has been indicated by Lynch et. al. (2006) that human salmonellosis is strongly related to foods of animal origin including beef products. In the United States, during the period between 1993 and 2002 a total of 274 foodborne illness outbreaks were linked to beef products and 23 (8.4%) were related to Salmonella , while Thorns (2000) concluded that bacterial food-borne zoonotic infections are the most common cause of human intestinal disorders. Salmonella account for over 90% of reported cases of bacterial food poisoning world-wide.

Salmonella was detected in 4% of local chilled samples while all frozen samples were free of Salmonella, According to ES (3602/2013) for fresh meat and ES (1522/ 2005) for frozen meat, all meat samples must be free from Salmonella spp. to be accepted for human consumption. Almost the same results of fresh meat samples were reported by Rhoades et al. (2009), who detected in average 3.8% Salmonella (0.0–7.5%) on raw beef samples. While the results of frozen samples were consistent with those reported by Elnawawi et al. (2012) examined for Salmonella species Incidence which failed to be isolated from any of examined samples. The same results were obtained by Mansour and Basha (2009). On the other hand, *Salmonella Typhimurium* was isolated from the carcass swabs at a percentage of 2.75% in average by Hassanin et al. (2017). Higher results were detected by Ukut et al. (2010), they collected and studied ten duplicate samples of meat from two major markets in Nigeria and revealed that fresh meat are commonly contaminated with pathogenic bacteria , Salmonella spp. was detected in 11.1% of the samples.

5. CONCULSION

From the overall results, we can conclude that both fresh and frozen imported meat are considered as a significant source of bacteriological public health hazard related to some food poisoning bacterial. There is no significant difference between Chilled and frozen meat in terms of acceptance based on ABC and total coliform contamination. Also, both fresh and frozen meat showed a close percentage of *E. coli* contamination. However, frozen meat samples were free of Salmonella spp. The effective control of beef-borne pathogens requires a longitudinally integrated (meat chain-based) approach, use of Good Manufacturing Practice/Good Hygienic Practice (GMP/ GHP) and Hazard Analysis and Critical Control Point (HACCP) principles and responsibility acceptance from all the participants in the meat chain, in addition to an appropriate considerations regarding resources availability, technical possibilities, consumers' attitude and behaviors, and cost-benefit as well.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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