



Bacteriological quality of frozen minced meat in Menofia Governorate

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ABSTRACT

A total of one hundred random samples of frozen minced beef (340g of each) were collected from four different meat factories A,B,C and D their products marketed in Menofia Governorate(25 for each). Factory D, recorded the highest count of Aerobic plate count (APC), Enterobacteriaceae and coliform as $9.17 \times 10^5 \pm 2.03 \times 10^5$, $7.29 \times 10^4 \pm 1.10 \times 10^4$ and $3.13 \times 10^4 \pm 0.62 \times 10^4$ cfu/g were recorded, respectively. Followed by factory C that recorded $4.90 \times 10^5 \pm 0.58 \times 10^5$, $5.42 \times 10^4 \pm 0.83 \times 10^4$ and $1.89 \times 10^4 \pm 0.35 \times 10^4$ cfu/g, respectively. In addition, factory D recorded the highest pathogenic load of staphylococci and staphylococcus aureus with $4.65 \times 10^4 \pm 0.76 \times 10^4$ & $1.16 \times 10^4 \pm 0.24 \times 10^4$ cfu/g, respectively. Followed by factory C which recorded $2.33 \times 10^4 \pm 0.49 \times 10^4$ & $8.83 \times 10^3 \pm 1.91 \times 10^3$ cfu/g, respectively. The highest incidence of Escherichia coli, Salmonella and S.aureus in factory D were 36%, 28% & 68% followed by factory C were 24%, 20% & 52%, then factory B were 20%, 16% & 40%, finally factory A were 12%, 8% and 24%, respectively. Achieved results in the present study proved that minced meat samples at factory C and D were highly contaminated that may considered a reliable index of fecal contamination and improper handling during processing.

Keywords: Frozen minced meat, APC, Enterobacteriaceae, Coliform, E.coli, S.aureus.

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1. INTRODUCTION

Meat and meat products are sources of high quality protein and their amino acid composition usually compensate for shortcomings in the food. They supply easily absorbed iron and assist the absorption of iron from other foods, they also rich sources of B-complex vitamins (Speedy, 2003).

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. Moreover, the complete elimination of pathogens from food processing environments is a difficult, in part

because bacteria can attach to meat contact surfaces where they survive even after cleaning and disinfection (Yang et al., 2012). Aerobic plate count (APC) is the most reliable index of meat quality, sanitary processing and storage life of meat products (ICMSF, 1980). Enterobacteriaceae group has an epidemiological importance as some of its members are pathogenic and may cause serious infections and food poisoning. Moreover, the total number of Enterobacteriaceae considered as an indication of possible enteric contamination in

the absence of coliforms (Mercuri et al., 1978).

Escherichia coli and *Staphylococcus aureus* are the most important bacterial pathogens in beef meat and meat products that are responsible for food-borne infections (Abdaslam, 2014; Ezzat et al. 2014 and Saif-Marwa, 2015).

Contamination of minced meat with *Salmonella* is still considered a major problem in food hygiene (Vipham, 2012). Salmonellosis is still one of the major global causes of gastroenteritis in humans and animals (Grimont and Weil 2007). The failure of the food manufacture to assure the production and distribution of safe food products lead to many serious food borne diseases which cause considerable morbidity & mortality as well as reduction in economic production (Alexandratos, 2010). Therefore, this study was conducted to throw out a light on the bacterial quality of some processed meat products in processing plants in relation to different hygienic levels of these markets.

2. Materials and methods

2.1. Collection of samples:

One hundred samples (340g of each sample) of frozen minced beef were collected from four different meat factories (A, B, C & D) (25 of each) in Shibin El-kome, Al-Menofia Province, Egypt. All collected samples were put in sterile plastic bags, transferred under hygienic measures in ice box to the laboratory without undue delay and examined bacteriologically.

2.2. Preparation of samples :(ISO 6887-2003)

Twenty- five grams of the examined samples were stomached by using (stomacher 3, serial no.010410226, France) with 225ml of 0.1% sterile buffered peptone water to give a dilution of (1:10), from which decimal serial dilutions were prepared.

2.3. Bacteriological examination:

Determination of APC (APHA 2001), Coliform count (Feng et al 2002), Enterobacteriaceae count (ICMSF 1996), isolation and identification of *Escherichia coli* were carried by (ISO 16649-2001), *Salmonella* (ISO 6579: 2002) and *Staphylococcus aureus* (FDA 2001).

2.4. Statistical Analysis:

The obtained results were statistically analyzed by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

It is evident from the result recorded in table (1) that the APC counts (cfu/g) in the examined samples of frozen minced beef at different meat factories A, B, C and D were $7.85 \times 10^4 \pm 1.26 \times 10^4$, $2.31 \times 10^5 \pm 0.19 \times 10^5$, $4.90 \times 10^5 \pm 0.58 \times 10^5$ & $9.17 \times 10^5 \pm 2.03 \times 10^5$ cfu/g, respectively. Enterobacteriaceae counts were $8.05 \times 10^3 \pm 1.67 \times 10^3$, $1.14 \times 10^4 \pm 0.20 \times 10^4$, $5.42 \times 10^4 \pm 0.83 \times 10^4$ & $7.29 \times 10^4 \pm 1.10 \times 10^4$ cfu/g at different meat factories A, B, C and D, respectively. Coliform counts were $3.96 \times 10^3 \pm 0.48 \times 10^3$, $6.01 \times 10^3 \pm 1.12 \times 10^3$, $1.89 \times 10^4 \pm 0.35 \times 10^4$ & $3.13 \times 10^4 \pm 0.62 \times 10^4$ cfu/g at different meat factories A, B, C & D, respectively. *Staphylococcus* counts were $5.72 \times 10^3 \pm 0.91 \times 10^3$, $8.05 \times 10^3 \pm 2.10 \times 10^3$, $2.33 \times 10^4 \pm 0.49 \times 10^4$ & $4.65 \times 10^4 \pm 0.76 \times 10^4$. *Staphylococcus aureus* counts at different meat factories A, B, C & D were $9.29 \times 10^2 \pm 2.06 \times 10^2$, $3.51 \times 10^3 \pm 0.73 \times 10^3$, $8.83 \times 10^3 \pm 1.91 \times 10^3$ & $1.16 \times 10^4 \pm 0.24 \times 10^4$ cfu/g, respectively.

From the previous results, it's revealed that there are high significant differences between counts in different meat factories A, B, C & D ($P < 0.05$) as shown in table (2) results recorded that the incidence of *E.coli*, *Salmonella* and *S.aureus* in the examined samples of frozen minced beef were 12%, 8%

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& 24% in (A), 20%, 16% & 40% in (B), 24%, 20% & 52% in (C) and 36%, 28% & 68% in (D) meat factories, respectively. The data recorded in table (3) indicated that the incidence of *E.coli* Serotypes in the examined frozen minced meat samples were *O26:H11(EHEC)* (4%), *O111:H2 (EHEC)* (4%) and *O119:H6* (4%) in (A), *O26:H11 (EHEC)* (8%), *O91:H21(EHEC)*(4%), *O111:H2 (EHEC)* (4%) and *O125:H21 (ETEC)* (4%) in (B), *O26:H11 (EHEC)* (4%), *O55:H7 (EPEC)* (4%), *O111:H2 (EHEC)* (8%), *O114:H4 (EPEC)* (4%) and *O124 (EIEC)* (4%) in (C), *O26:H11(EHEC)* (12%), *O86 (EPEC)* (4%), *O91:H21 (EHEC)* (4%), *O111:H2 (EHEC)* (8%), *O119:H6 (EPEC)* (4%) and *O128:H2 (ETEC)* (4%)

in (D). On the other hand, results in table (4) showed that the incidence of *Salmonella* serotypes in the examined samples were *S. Enteritidis*(4%) and *S.Montevideo*(4%) in (A), *S.Enteritidis* (4%), *S.Infantis* (4%) and *S.Typhimurium* (8%) in (B), *S.Heidllberg* (4%), *S.Montevideo* (8%), *S.Molade* (4%) and *S.Typhimurium* (4%) in (C), *S.Enteritidis* (8%), *S.Infantis* (4%), *S.Molade* (4%), *S.Tamale* (4%) and *S.Typhimurium* (8%) in (D). Table (5) declared that all the examined samples of minced meat in factory(A) were accepted, 4%, 16% & 36%, 12%, 20%, 24% & 36%, 8%, 12%, 20% & 28% and 24%, 40%, 52% & 68% were unaccepted for *E.coli*, *Salmonella* and *S.aureus* in (A), (B), (C) & (D) meat factories according to E.S. (2005).

Table1: Mean values of bacterial counts (cfu/g) in the examined frozen minced beef samples from four different meat factories (n=25).

Meat factories counts	A	B	C	D
APC	7.85X10 ⁴ ±1.26X10 ⁴	2.31X10 ⁵ ±0.19X10 ⁵	4.90X10 ⁵ ±0.58X10 ⁵	9.17X10 ⁵ ±2.03X10 ⁵
Enterobacteriaceae	8.05x10 ³ ±1.67x10 ³	1.14x10 ⁴ ± 0.20x10 ⁴	5.42x10 ⁴ ±0.83x10 ⁴	7.29x10 ⁴ ± 1.10x10 ⁴
Coli form	3.96x10 ³ ±0.48x10 ³	6.01x10 ³ ± 1.12x10 ³	1.89x10 ⁴ ± 0.35x10 ⁴	3.13x10 ⁴ ±0.62x10 ⁴
Staphylococcus	5.72x10 ³ ±0.91x10 ³	8.05x10 ³ ±2.10x10 ³	2.33x10 ⁴ ±0.49x10 ⁴	4.65x10 ⁴ ±0.76x10 ⁴
Staph.aureus	9.29x10 ² ± 2.06x10 ²	3.51x10 ³ ±0.73x10 ³	8.83x10 ³ ±1.91x10 ³	1.16x10 ⁴ ± 0.24x10 ⁴

Highly significant difference between products at P<0.01.

Table 2: Incidence of contamination in the examined minced meat samples (n=25).

Meat factories counts	A		B		C		D	
	NO.	%	NO.	%	NO.	%	NO.	%
APC	0	0	1	4	4	16	9	36
E.coli	3	12	5	20	6	24	9	36
Salmonella	2	8	4	16	5	20	7	28
Staph.aureus	6	24	10	40	13	52	17	68

Table 3: Serotyping of *E.coli* in the examined frozen minced meat at different meat factories (n=25)

Meat factories E.coli strains	A		B		C		D		Strains characteristics
	NO.	%	NO.	%	NO.	%	NO.	%	
<i>O26:H11</i>	1	4	2	8	1	4	3	12	EHEC
<i>O55:H7</i>	-	-	-	-	1	4	-	-	EPEC
<i>O86</i>	-	-	-	-	-	-	1	4	EPEC
<i>O91:H21</i>	-	-	1	4	-	-	1	4	EHEC
<i>O111:H2</i>	1	4	1	4	2	8	2	8	EHEC
<i>O114:H4</i>	-	-	-	-	1	4	-	-	EPEC
<i>O119:H6</i>	1	4	-	-	-	-	1	4	EPEC
<i>O124</i>	-	-	-	-	1	4	-	-	EIEC
<i>O125:H21</i>	-	-	1	4	-	-	-	-	ETEC
<i>O128:H2</i>	-	-	-	-	-	-	1	4	ETEC
Total	3	12	5	20	6	24	9	36	

Table 4: Serotyping of Salmonella in the examined frozen minced meat at different meat factories (n=25).

Meat factories Salmonellae	A		B		C		D		Group	Antigenic O	Structure H
	NO.	%	NO.	%	NO.	%	NO.	%			
<i>S. Enteritidis</i>	1	4	1	4	-	-	2	8	D1	1,9,12	g.m:1,7
<i>S. Heidelberg</i>	-	-	-	-	1	4	-	-	B	1,4,5,12	r:1,2
<i>S. Infantis</i>	-	-	1	4	-	-	1	4	C1	6,7	r:1,5
<i>S. Montevideo</i>	1	4	-	-	2	8	-	-	C1	6,7	g.m.s:1,2,7
<i>S. Molade</i>	-	-	-	-	1	4	1	4	C1	6,7	Z10:z6
<i>S. Tamale</i>	-	-	-	-	-	-	1	4	C3	8,20	Z29:e,n,Z15
<i>S. Typhimurium</i>	-	-	2	8	1	4	2	8	B	1,4,5,12	I:1,2
Total	2	8	4	16	5	20	7	28			

Table 5: Acceptability of bacterial load in the examined samples of frozen minced meat according to permissible limits of E.S. (2005).

m.o.s meat factories	APC		E.coli		Salmonella		S.aureus	
	Unaccepted		Unaccepted		Unaccepted		Unaccepted	
	P.L	Samples No. %	P.L	Samples No. %	P.L	Samples No. %	P.L	Samples No. %
A	10 ⁶	0 0	free	3 12	free	2 8	10 ²	6 24
B	10 ⁶	1 4	free	5 20	free	4 12	10 ²	10 40
C	10 ⁶	4 16	free	6 24	free	5 20	10 ²	13 52
D	10 ⁶	9 36	free	9 36	free	7 28	10 ²	17 68

4. DISCUSSION

Minced meat and meat products are perishable foods and unless stored under proper conditions spoil quickly. In addition, if pathogens are present, meat products become hazardous for consumers. Therefore, assurance of meat safety and quality is the most important (Shimoni and Iabuzza, 2000).

Results demonstrated agreed with those of (Biswas et al., 2008), where they reported that minced meat of high incidences to bacterial contamination. The clothes of workers, processing equipment and water used to wash carcass, hands and equipment were source of meat contamination during slaughter process (Upmann et al, 2000).The sources could be the animal, the environment or contamination during meat processing (McNamara 1998). The main sources of meat contamination are animal feces especially during processing at the slaughterhouse (Kudva et al., 1998).

The results revealed in table (1) reflected that the highest APC, *Enterobacteriaceae*, *Coliform*, *Staphylococcus* and *S.aureus* were

in the examined frozen minced meat collected at meat factory (D), followed by (C) then (B) and finally(A) meat factories. High APC may be attributed to the contamination of the product from different sources or unsatisfactory processing as well as unsuitable condition during storage Zehrandalia (2008).

In general, *Enterobacteriaceae* count used to assess the general hygienic status of a food product and their presence in food indicates in adequate cooking or post-processing contamination (CFS, 2014). Members of this family have been considered a potent cause of food borne outbreaks (Centinkaya et al., 2008).

Coliform counts reflect inadequate sanitation during production and handling raw material, meat contact surfaces and employees. Meanwhile, the occurrences of large numbers of them on carcass surfaces are highly undesirable, mostly suggesting faecal contamination and points to potentially severe hazard (Eribo and Jay, 1985).

Presence of *Staphylococci* in minced meat samples may be attributed to inadequate heat treatment, unhygienic handling practices through the workers who can transfer staphylococci on their hands and using dirty equipment's for slicing and the most probable reason of minced meat contamination might be the poor hygienic quality of raw meat, inadequate storage and thawing conditions, contamination from grinder and the time between mincing and mixing Eisel et al. (1997).

According to results achieved in table(1) comparing the obtained values from the examined samples, higher result for APC, *Coliform* and *Staphylococci* count were reported by Tekinsen et al.(1980) (8.4×10^7 cfu/g), Gonulalan and Kose (2003) (5.3×10^9 cfu/g) and Baskaya et al. (2004) (6.3×10^7 cfu/g) in minced meat in APC. While, Erdem et al. (2014) who found that *Coliform* counts (cfu/g) of minced meat were (1×10^6 cfu/g). Also, higher ones of *Staphylococci* showed by Ibrahim (2016) ($3.00 \times 10^7 \pm 8.13 \times 10^6$ cfu/g) & ($5.83 \times 10^5 \pm 1.06 \times 10^5$ cfu/g) and Talaat (2009) ($6.92 \times 10^6 \pm 4.54 \times 10^6$ cfu/g) in frozen minced meat.

On the other hand, lower results for APC were reported by Melngaile et al., (2014) ($5.08 \log$ cfu/g) in minced meat. Ahmed (2018) revealed that *Enterobacteriaceae* counts varied from 4.0×10^2 to 6.4×10^4 with mean value of $5.82 \times 10^3 \pm 1.02 \times 10^3$ cfu/g in minced meat. Moreover, Morshdy et al. (2013) (4.3×10^2 /g) and Erdem-Ayten et al. (2014) (6.5×10^2 /g) for *S.aureus* in minced meat.

While, it is evident from the results in Table (1) that there is nearly similar results obtained by Amany et al. (2010) (5.61×10^5 cfu/g) in minced meat for APC. In addition, Al-Mutairi (2011) revealed that *Enterobacteriaceae* count were (37.8×10^4 cfu/g) in minced meat. The obtained results of *Coliform* in (A) level

were relatively similar to Al-kour (2001) (2.34×10^3 cfu/g) & Amany et al. (2010) (5.12×10^3 cfu/g) in minced meat.

Table (2) revealed that high incidence of APC, *E.coli*, *Salmonella* and *S.aureus* at (D) and (C) but the lowest one in (A). Presence of *E.coli* in meat indicated a general lack of cleanness during slaughtering, evisceration, dressing, transportation and handling of meat ICMSF(1996b).As well as, *E.coli* may be used as an indicator microorganism of fecal contamination and poor sanitation during processing (Eisel et al.1997). Also, *Salmonella* % at (B) were nearly similar results obtained by Stock and Stolle (2001) (15.8%) and Ejeta et al. (2004) (14.4%) but % at (A) is nearly similar with White et al. (2001) (9%).Higher results founded by Fritzen et al. (2006) (48%) and Ahmed (2012) (40%). On the other hand, the lowest % reported by Fathi and Thabet (2001) (0%). Presence of *Salmonella* may reflect insufficient hygienic measures stock and Stolle (2001).

Presence of *S.aureus* is a leading cause of food poisoning resulting from the consumption of contaminated food with Staphylococcal enterotoxins Guven et al. (2010).

Table (3) showed that serotyping of *E.coli* which found the highest rate of contamination in (D) followed by (C) then(B) but the lowest one in (A).*Escherichia coli*% recorded in (C) were nearly similar to those obtained by Melngaile et al. (2014) (23%). While, higher ones (46%) were obtained by Shawish (2015). *Enterohemorrhagic Escherichia coli* (EHEC) was implicated in outbreaks of diarrhea in young children and infants. Illness caused by EHEC is typically quit severe and characterized by sudden onset of severe crampy abdominal pain followed by watery diarrhea, which later on becomes grossly bloody, Lee et al. (2009).

As shown in Table (4) salmonella serotyping found high contamination in (D) then (C) then (A) but lowest one at (A). *S.Typhimurium* has been the most frequent serotype and *S. Enteritidis* acts as a causative agent of human gastroenteritis throughout the world Sharma et al., 1996).

As shown in Table (5) results indicated that all examined minced meat samples in (A) were accepted, but APC in (B, C &D) were unaccepted. Also, *E.coli*, *Salmonella* and *S.aureus* in examined samples at A, B, C &D factories more than permissible limits of E.S(2005) in such meat products represent a high risk to consumer, cause health hazard and indicates inadequate sanitary conditions during stages of manufacturing, dirty equipment and improper handling.

5. Conclusion

Achieved results in the present study proved that the highest contaminated minced meat samples were collected in (D) factory followed by (C) that may considered a reliable index of fecal contamination and improper handling during processing.

Consequently, strict maintenance of good practices during processing, strengthened by maintaining the cold chain during transport, distribution and carcass commercialization is of central importance to ensure both public health and food quality.

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