

Bacteriological Evaluation of Frozen Sausage

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Abstract

105 random samples of frozen sausages, collected from different localities (Benha city, Centers of kaliobia governorate and villages) with 35 for each locality to evaluate the bacterial quality and the hygienic health hazard of them. The mean value of APC, Psychrotrophic, Enterobacteriaceae, Coliform and Staphylococcus counts in frozen sausage samples collected from Benha city were $3.62 \times 10^4 \pm 0.19 \times 10^4$; $2.10 \times 10^4 \pm 0.13 \times 10^4$; $1.53 \times 10^2 \pm 0.09 \times 10^2$; $0.92 \times 10^2 \pm 0.13 \times 10^2$ and $1.62 \times 10^2 \pm 0.10 \times 10^2$, respectively; for frozen sausage samples collected from different centers of kaliobia governorate were $5.88 \times 10^4 \pm 0.17 \times 10^4$; $2.98 \times 10^4 \pm 0.11 \times 10^4$; $2.36 \times 10^2 \pm 0.11 \times 10^2$; $1.93 \times 10^2 \pm 0.10 \times 10^2$ and $2.10 \times 10^2 \pm 0.09 \times 10^2$, respectively; for frozen sausage samples collected from different villages were $7.91 \times 10^4 \pm 0.16 \times 10^4$; $5.32 \times 10^4 \pm 0.22 \times 10^4$; $4.84 \times 10^2 \pm 0.24 \times 10^2$; $4.33 \times 10^2 \pm 0.22 \times 10^2$ and $3.23 \times 10^2 \pm 0.16 \times 10^2$, respectively. SET- RPLA test revealed that, seven strains out of 10 random examined strains (70.0%) were enterotoxigenic and classified according to type of toxin into (4A; 1B; 2A & C).

Keywords: Sausages; Bacteriological evaluation; SET_RPLA test; E. Coli-S.aureus

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Introduction

Sausages are popular meat products enjoyed by millions of consumers worldwide. It consider one of the most traditional meat products in Egypt and it is mostly produced from beef meat, fat tissues, dry rusk, salt and spices. Consumers are increasingly preferring products with low fat content while retain good flavor, quick easily prepared meat meals, solve the problem of the shortage in fresh meat of high price which is not reach to large numbers of families with limited income homes, lack of their time available for preparation of family meals and overall acceptability (Lin and Huang, 2008 and Abd-El Salam 2014).

Freezing are widely, quickly and effectively used method for preserving meat and meat products than canning and drying. It has been practiced for several years to maintain its quality during storage, distribution and marketing. Although, meat freezing is generally conceded to cause tissue damage and some quality loss, it remains the method of preference for long-term storage in North America and the

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exposure of frozen meat to thawing and refreezing in market shops and street vendors must be avoided as it yielding an abundant supply of water and form an excellent medium for microbial growth and multiplication (Abd El-Aziz, 2000). Microbiological criteria are a useful way to determine the safety and quality of sausage and the presence of food poisoning microorganisms in sausage depends on the meat used for mincing, sanitary conditions and practices in preparation time, temperature of storage and transportation resulting in an inferior or even unfit quality for human consumption. (Mantis., *et al.* 2005; Ercolini., *et al.* 2006 and Akhtar., *et al.* 2014).

Contaminated frozen beef and meat products may constitute a public health hazard (Hamed., *et al.* 2015). The most important bacterial pathogens in beef meat and meat products that are responsible for foodborne infections include *E. coli*, Salmonellae, coagulase positive *S.aureus* and Pseudomonas (Farag and Korashy-Nahla, 2008 and Hamed., *et al.* 2015). Bacterial contamination and hygienic measures during meat production and bad storage conditions for frozen meat products can be measured using the aerobic plate count, total psychrotroph counts, total Enterobacteriaceae and total Coliforms (Zweifel., *et al.* 2005 and Hamed., *et al.* 2015). Pseudomonas spp. are the most important Psychotropics spoilage organisms associated with meat and meat products and the presence of them lead to unsafe food (Hillers., *et al.* 2003 and Farag and Korashy- Nahla, 2008).

Also, they considered as nosocomial pathogen of immune compromised individuals (Todars., *et al.* 2004). The presence of *E. Coli* in food generally indicates direct and in direct fecal contamination (Clarence., *et al.* 2009). It is commonly non-virulent but some strains have adopted pathogenic or toxigenic virulence factors that make them virulent to human and animals. It has become recognized as a serious food borne pathogen and has been associated with numerous out breaks of disease resulting from contaminated beef and meat products (Kaper., *et al.* 2004 and Gi., *et al.* 2009).

Staphylococcal intoxication is a leading cause of foodborne intoxication and enterotoxigenic Staphylococcus strains have been isolated from foods implicated in illnesses (Cencil., *et al.* 2003). *S. aureus* is considered to be one of the most important foodborne disease worldwide due to its ability to produce wide variety of toxins (Argudin., *et al.* 2010). The Staphylococcal enterotoxins (SEs) are responsible for the symptoms that associated with Staphylococcal food poisoning (Llewelyn and Cohen, 2002). The disease is characterized by symptoms including nausea, vomiting, abdominal cramps and diarrhea lasting from 24 to 48h and the complete recovery usually occurs within 1-3 days.

Recent food surveys confirmed that Aeromonas spp. were considered as re- emerging enteric pathogens that responsible for several food borne illness and outbreaks (Ghenghesh., *et al.* 2008). As information on microbiology of frozen sausage in Egypt is very limited; therefore, the present study was conducted to evaluate the safety and quality of frozen sausage at different localities in Kaliobia Governorate by studying the bacterial status of them.

Material and Methods

Samples collection

A total of 105 random samples of frozen sausages, were collected from different localities (Benha city, Centers of kaliobia governorate and villages with 35 for each locality) at Kaliobia Governorate. Each sample was kept in a separate sterile plastic bag and put in an icbox then transferred to the laboratory under complete aseptic conditions without undue delay and examined bacteriologically to evaluate the bacterial quality and the hygienic health hazard of them with some food borne pathogens

Bacteriological examination

1. Preparation of samples (APHA, 2001).
2. Determination of Aerobic Plate Count (APC)/gram, using the standard plate count following (FDA, 2001).
3. Determination of Total Psychrotrophic count (APHA, 2001)

4. Determination of Total Enterobacteriaceae count using the surface plating method of ICMSF, 1996 using Violet Red Bile Glucose agar medium (VRBG). The plates were incubated at 37°C for 24 hours. All purple colonies were then counted and the total number of colonies was determined. Hence, the Enterobacteriaceae count/g was calculated and recorded.
5. Total Coliform count using the surface plating method of ICMSF, 1996 using Violet Red Bile agar medium.
6. Isolation and identification of *E. Coli* following (ISO, 2001): Typical *E. Coli* colonies (pink - orange colonies) were picked up for identification morphologically by Gram stain; biochemically, serologically by slide agglutination test (using *E. Coli* antisera "SEIK-EN" Set 1, consists of 8 polyvalent and 43 (OK) antisera of DENKA SEIKEN Co. LTD. Tokyo, Japan) following (Edward and Ewing 1972 and Quinn., *et al.* 2002) and In-Vitro anti-microbial sensitivity test for isolated *E.coli* according to (Koneman., *et al.* 1997).
7. Determination of Total Staphylococci Count following (ICMSF, 1996). Isolation of *S.aureus* using Baird-Parker Agar Plates. Suspected colonies were picked up onto slants of nutrient agar for further purification then identified morphologically by Gram-stain; biochemically and coagulase activities according to ICMSF (1996) and Quinn., *et al.* (2002). Detection of Enterotoxins producing isolates by SET- RPLA technique (Igarashi., *et al.* 1986) and *In-Vitro* anti-microbial sensitivity test for isolated *S.aureus* according to (Koneman., *et al.* 1997).
8. Isolation and identification of *Pseudomonas* species following (Quinn., *et al.* 2002 and Markey., *et al.* 2013).
9. Isolation of *Aeromonas* species following (Quinn., *et al.* 2002 and Nicky, 2004).
10. Isolation and identification of *Salmonella* following (ISO, 2002). Suspected *Salmonella* colonies that appeared as red with black centers on XLD agar and pink on Brilliant Green agar were identified morphologically by Gram-stain and biochemically according to (Quinn., *et al.* 2002).
11. Data obtained were analyzed according to Snedecor and Cochran (1969) using the computer software program (SPSS, 2001).

Results

The results of bacteriological examination of frozen sausage samples collected from different areas at Kaliobia Governorate (Benha city, different centers and different villages) are presented in Tables (1-11). *A. hydrophila* strains were the only species isolated from examined samples. Only three frozen sausage samples collected from different villages were positive for *A. hydrophila* isolation, meanwhile, they failed to be detected in all examined samples of frozen sausage collected from Benha city and different centers at Kaliobia Governorate. Only two frozen sausage samples collected from different villages were positive for *Salmonella* spp. isolation, meanwhile, they failed to be detected in all examined samples of frozen sausage collected from Benha city and different centers at Kaliobia Governorate.

Sample area	Negative		Positive		Min.	Max.	Mean ± SEM**
	No.	%*	No.	%*			
Benha city	0	0.0	35	100.0	1.1×10 ⁴	5.9×10 ⁴	3.62 × 10 ⁴ ± 0.19 × 10 ⁴ ^c
Centers	0	0.0	35	100.0	3.8 × 10 ⁴	7.5×10 ⁴	5.88 × 10 ⁴ ± 0.17 × 10 ⁴ ^b
Villages	0	0.0	35	100.0	4.8×10 ⁴	9.9×10 ⁴	7.91 × 10 ⁴ ± 0.16 × 10 ⁴ ^a

*Percentage in relation to total number of sample in each row.

**Standard error of mean

***As all examined samples of frozen Sausage collected from different areas were lower than 105, so all samples were accepted following ES (2005).

Table 1: Aerobic plate count /g. (APC) in the examined samples of frozen sausage (n=35 for each sample).

Sample area	Negative		Positive		Min.	Max.	Mean \pm SEM**
	No.	%*	No.	%*			
Benha city	4	11.4	31	88.6	0.7×10^4	3.4×10^4	$2.10 \times 10^4 \pm 0.13 \times 10^{2c}$
Centers	3	8.6	32	91.4	1.9×10^4	4.4×10^4	$2.98 \times 10^4 \pm 0.11 \times 10^{4b}$
Villages	0	0.0	35	100.0	3.2×10^4	8.0×10^4	$5.32 \times 10^4 \pm 0.22 \times 10^{4a}$

*Percentage in relation to total number of sample in each row.

**Standard error of mean

***As all examined samples of frozen sausage collected from different areas were lower than 105, so all samples were accepted following ES (2005).

Table 2: Total Psychrotrophic count/g. in the examined samples of frozen sausage (n = 35 for each sample).

Sample area	Negative		Positive		Min.	Max.	Mean \pm SEM**
	No.	%*	No.	%*			
Benha city	11	31.4	24	68.6	0.8×10^2	2.7×10^2	$1.53 \times 10^2 \pm 0.09 \times 10^{2c}$
Centers	7	20.0	28	80.0	1.6×10^2	3.8×10^2	$2.36 \times 10^2 \pm 0.11 \times 10^{2b}$
Villages	2	5.7	33	94.3	1.9×10^2	6.7×10^2	$4.84 \times 10^2 \pm 0.24 \times 10^{2a}$

*Percentage in relation to total number of sample in each row.

**Standard error of mean

***The statistical results revealed that, frozen Sausage samples collected from different villages showed a significant ($P \leq 0.05$) increase of Enterobacteriaceae counts when compared with other samples.

Moreover, there were no significant difference ($P > 0.05$) of Enterobacteriaceae counts between frozen sausage samples collected from Benha city and frozen sausage samples collected from different centers.

Table 3: Enterobacteriaceae count/g. in the examined samples of frozen sausage (n=35 for each sample).

Sample area	Negative		Positive		Min.	Max.	Mean \pm SEM**
	No.	%*	No.	%*			
Benha city	23	65.7	12	34.3	0.4×10^2	2.1×10^2	$0.92 \times 10^2 \pm 0.13 \times 10^{2c}$
Centers	12	34.3	23	65.7	1.1×10^2	2.9×10^2	$1.93 \times 10^2 \pm 0.10 \times 10^{2b}$
Villages	4	11.4	31	88.6	2.4×10^2	6.5×10^2	$4.33 \times 10^2 \pm 0.22 \times 10^{2a}$

*Percentage in relation to total number of sample in each row

**Standard error of mean

Table 4: Coliforms count/g. in the examined samples of frozen sausage (n = 35 for each sample).

Sample area	No.	Positive		No. of accepted samples**	No. of non-accepted samples**
		No.	%*		
Benha city	35	3	8.6	32	3
Centers	35	5	14.3	30	5
Villages	35	8	22.9	27	8
Total	105	16	15.2	89	16

*Percentage in relation to total number of sample in each row.

**Accepted and non- accepted samples according to (ES, 2005)

Table 5: Incidence of *E.coli* in examined samples of frozen sausage ($n = 35$ for each sample).

Sample area	Benha city		Centers		Villages		Strain Characteristic
	No.	%*	No.	%*	No.	%*	
<i>E.coli</i> serotype							
O55:K59(B5)	1	2.86	2	5.71	3	8.57	EPEC
O125:K59(B5)	1	2.86	1	2.86	3	8.57	EPEC(EPEC)
O111:K58(B9)	0	0.0	1	2.86	1	2.86	EHEC
O126:K71(B16)	1	2.86	1	2.86	1	2.86	EPEC
Total	3	8.57	5	14.28	8	22.86	-

*Percentage in relation to total number of each sample (35). EPEC: Enteropathogenic *E.coli*

EPEC: Enterotoxigenic *E.coli*

EHEC: Enterohaemorrhagic *E.coli*

Table 6: Incidence and serotyping of *E.coli* isolated from positive samples of frozen sausage ($n = 35$ for each sample).

Sample area	Negative		Positive		Min.	Max.	Mean \pm SEM**
	No.	%*	No.	%*			
Benha city	9	25.7	26	74.3	0.8×10^2	2.6×10^2	$1.62 \times 10^2 \pm 0.10 \times 10^{2c}$
Centers	5	14.3	30	85.7	1.2×10^2	3.3×10^2	$2.10 \times 10^2 \pm 0.09 \times 10^{2b}$
Villages	3	8.6	32	91.4	2.2×10^2	6.1×10^2	$3.23 \times 10^2 \pm 0.16 \times 10^{2a}$

*Percentage in relation to total number of sample in each row

**Standard error of mean

Table 7: Staphylococci count/g. in the examined samples of frozen sausage ($n = 35$ for each sample).

Sample	No.	Positive		No. of accepted samples**	No. of non- accepted samples**
		No.	%*		
Benha city	35	5	14.3	30	5
Centers	35	7	20.0	28	7
Villages	35	10	28.6	25	10
Total	105	22	20.9	83	22

* Percentage in relation to total number of sample in each row.

**Accepted and non- accepted samples according to (ES, 2005) in relation to the isolation of Coagulase Positive *S. aureus*.

The results of SET -RPLA test revealed that, 7 strains out of 10 random examined strains (70.0%) were enterotoxigenic and classified according to type of toxin into (4A; 1B;2A&C).

Ps. aeruginosa strains were the only species isolated from examined samples.

Table 8: Incidence of Coagulase Positive *S. aureus* in examined samples of frozen sausage (n = 35 for each sample).

Sample area	No.	Positive		No. of accepted samples**	No. of non- accepted samples**
		No.	%*		
Benha city	35	2	5.7	33	2
Centers	35	3	8.6	32	3
Villages	35	6	17.1	29	6
Total	105	11	10.5	94	11

* Percentage in relation to total number of sample in each row.

**Accepted and non- accepted samples according to (ES, 2005) in relation to the isolation of *Ps.aeruginosa*.

Table 9: Incidence of *Ps. aeruginosa* strains in examined samples of frozen sausage (n=35 for each sample).

Discussion

Sausages are considered as an ideal culture medium for growth of many microorganisms as *E. Coli*; *Salmonella*; *Staph.aureus*; *Pseudomonas*; *Micrococcus*; *Lactobacillus* and *Aeromonas* resulting in their spoilage, economic losses, foodborne infections in human and health risk (Ercolini, *et al.* 2006 ; Akhtar, *et al.* 2014 and Hamed., *et al.* 2015).

The results of APC counts Table (1) revealed that, the minimum and maximum counts (APC) in the examined frozen sausage samples collected from different localities (Benha city, centers and villages) were ranged from 1.1×10^4 to 5.9×10^4 ; 3.8×10^4 to 7.5×10^4 and 4.8×10^4 to 9.9×10^4 respectively, with a mean value of $3.62 \times 10^4 \pm 0.19 \times 10^4$; $5.88 \times 10^4 \pm 0.17 \times 10^4$ and $7.91 \times 10^4 \pm 0.16 \times 10^4$, respectively. However, the counts were considered satisfactory, as these results were lower than those suggested by ES (2005). Nearly similar counts were recorded by Gibriel, *et al.* (2007); El-Maghraby-Marwa (2014); Mousa., *et al.* (2014) ; Hamed., *et al.* (2015) and Heweidly (2017). The results in Table (2) appeared that, the minimum and the maximum Psychrotrophic count in the examined frozen sausage samples collected from different localities (Benha city, centers and villages) were ranged from 0.7×10^4 to 3.4×10^4 ; 1.9×10^4 to 4.4×10^4 and 3.2×10^4 to 8.0×10^4 respectively, with a mean value of $2.10 \times 10^4 \pm 0.13 \times 10^4$; $2.98 \times 10^4 \pm 0.11 \times 10^4$ and $5.32 \times 10^4 \pm 0.22 \times 10^4$, respectively. As all positive samples of frozen sausage collected from different areas were lower than 105, so all samples were accepted following ES (2005). These results were agree with those of Mahmoud (2001); Karaboz and Dincer (2002) and Sharoba (2009). The results in Table (3) appeared that, the minimum and the maximum Enterobacteriaceae count in the examined frozen sausage samples collected from different localities (Benha city, centers and villages) were ranged from 0.8×10^2 to 2.7×10^2 ; 1.6×10^2 to

3.8×10^2 and 1.9×10^2 to 6.7×10^2 respectively, with a mean value of $1.53 \times 10^2 \pm 0.09 \times 10^2$; $2.36 \times 10^2 \pm 0.11 \times 10^2$ and $4.84 \times 10^2 \pm 0.24 \times 10^2$, respectively.

These results were in agreement with those of Otaiza, *et al.* (2006); Stagnitta, *et al.* (2006); Al-Mutairi (2011) and Heweidy (2017). Enterobacteriaceae have an epidemiological importance and the presence of them in meat indicates a microbial proliferation, as some of their members are pathogenic and may cause serious infections and food poisoning outbreaks to human being (Mosupye and Van Holy, 2000). The presence of coliforms in food indicates poor hygienic standards. Data presented in Table (4) showed that, the minimum and the maximum Coliform count in the examined frozen sausage samples collected from different localities (Benha city, centers and villages) were ranged from 0.4×10^2 to 2.1×10^2 ; 1.1×10^2 to 2.9×10^2 and 2.4×10^2 to 6.5×10^2 respectively, with a mean value of $0.92 \times 10^2 \pm 0.13 \times 10^2$; $1.93 \times 10^2 \pm 0.10 \times 10^2$ and $4.33 \times 10^2 \pm 0.22 \times 10^2$, respectively. These results came in parallel with those of Stagnitta, *et al.* (2006); Gibriel, *et al.* (2007); Al-Mutairi (2011); El-Maghraby-Marwa (2014); Mousa, *et al.* (2014); Hamed, *et al.* (2015) and Heweidy (2017).

Meanwhile, these results were disagreed with those of Abou-Aly, *et al.* (2007) and Abd El-Aziz- wafaa, (2015) who recorded higher coliform counts. The isolation of *E. Coli* from meat samples indicates fecal contamination and implies that other pathogens of fecal origin may be present. The increased incidence of *E. Coli* in the examined samples may be due to mishandling during production, processing and distribution or to the use of contaminated water during evisceration and slaughtering (Aycicek, *et al.* 2004 and Gwida, *et al.* 2014). The results in Tables (5 & 6) revealed that, 16 isolates of *E. Coli* were isolated from examined frozen sausage samples collected from different localities (Benha city, centers and villages) represented as 3(8.6%) from samples of Benha city with serotypes one 055:K59 (B5); one 0125:K59 (B5) and one 0126:K71 (B16); 5 (14.3%) from samples of centers with serotypes 2 055:K59(B5) , one 0125:K59(B5), one 0111:K58 (B9) and one 0126:K71(B16) and 8 (22.9%) from samples of villages with serotypes 3 055:K59 (B5), 3 0125:K59 (B5), one 0111:K58(B9) and one 0126:K71(B16). Nearly similar results were obtained by Kalantari, *et al.* (2012); Mansour (2013); El-Maghraby-Marwa (2014); Ezzat, *et al.* (2014); Abd El-Tawab, *et al.* (2015); Tarabees, *et al.* (2015); Armany *et al.* (2016) and Heweidy (2017). Meanwhile, these results were disagreed with those of Zaki-Eman (2003); Abou Hussein-Reham (2004); Abdaslam, *et al.* (2014); Sobieh(2014) and Ramadan (2015) who isolated *E.coli* from sausage samples with high incidence. Also, disagreed with Siriken, *et al.* (2006) and Hamed, *et al.* (2015) who failed to isolate *E. Coli* from sausage samples. Moreover, the same serotypes of *E. Coli* were previously isolated by Maarouf and Nassif-Marionette (2008); Mansour (2013); Abd El-Tawab, *et al.* (2015); Shawish (2015) and Tarabees, *et al.* (2015).

Moreover, these results coincided with the fact of Woody, *et al.* (1998) who recorded that the same serogroups were Enteropathogenic *E. Coli* and causing infantile enteritis; haemorrhagic colitis; haemorrhagic gastroenteritis and diarrheal illness in different settings.

The results of total Staphylococcus counts Table (7) revealed that, the minimum and the maximum Staphylococcus counts in the examined frozen sausage samples collected from different localities (Benha city, centers and villages) were ranged from 0.8×10^2 to 2.6×10^2 ; 1.2×10^2 to 3.3×10^2 and 2.2×10^2 to 6.1×10^2 respectively, with a mean value of $1.62 \times 10^2 \pm 0.10 \times 10^2$; $2.10 \times 10^2 \pm 0.09 \times 10^2$ and $3.23 \times 10^2 \pm 0.16 \times 10^2$, respectively.

These counts came in agreement with Hamouda (2005); Gibriel, *et al.* (2007); Ahmed- Alyaa (2015) and Heweidy (2017). Meanwhile, the results disagreed with those of El-Mossalami (2003); Zaki-Eman (2003); El-Ghamry (2004); Oluwafemi and Simisaye (2006); Ibrahim-Eman (2008); Sharoba (2009); El-Maghraby Marwa (2014) and Abd El-Aziz- wafaa, (2015) who reported higher Staphylococcus counts in examined sausage samples and with Sachindra, *et al.* (2005) who failed to isolate Staphylococcus from them. Moreover, the results revealed that, samples collected from different villages showed high counts when compared with other samples. This may be due to the combination of the low quality of sausage sold; poor manufacturing processes; inadequate cleaning and disinfection of both equipment and surfaces or poor personal hygiene; use of untrained personnel and long storage periods with periodical cutting of electric wires or using inconstant power of electric supply with fuel powered generating sets, that leading to frequent thawing and freezing of products

in villages resulting in an inferior or even unfit quality for human consumption. (Ercolini, *et al.* 2006; Adesiji, *et al.* 2011 and Akhtar, *et al.* 2014). The results obtained in Table (7) revealed that, 22 isolates of Coagulase positive *S.aureus* were isolated from examined frozen beef sausage samples represented as 5(14.3%) from samples of Benha city; 7(20.0%) from samples of centers and 10(28.6%) from samples of villages. These results came in accordance with those obtained by Ouf - Jehan (2001); Soultos, *et al.* (2003); Ibrahim-Eman (2008); Maarouf and Nassif-Marionette (2008); Abdel-Raouf, *et al.* (2014); Sobieh (2014); Abd El-Tawab, *et al.* (2015); Tarabees, *et al.* (2015); Armany, *et al.* (2016) and Heweidy (2017).

Meanwhile, these results were disagreed with those of El-Mossalami (2003); Hassanien-Fatin (2004); Gergis (2005); Benzerra, *et al.* (2010); Abdaslam, *et al.* (2014); El-Maghraby-Marwa (2014) and Mousa, *et al.* (2014) who isolated *S. aureus* from beef sausage samples with high incidence.

Also, disagreed with Otaiza, *et al.* (2006) and Kalantari, *et al.* (2012) who failed to isolate *S. aureus* from frozen beef sausage samples. The presence of *S. aureus* in meat and its products indicates poor hygiene of meat handlers as well as lack of sterilization of utensils and they grow without pronounced change in odour or taste in the products and producing heat stable enterotoxins which lead to food poisoning with severe diarrhoea and gastroenteritis among consumers (Protocarrero, *et al.* 2002 and Plaatjes, *et al.* 2004). The results of SET- RPLA test appeared that, 7 strains out of 10 random examined strains (70.0%) were enterotoxigenic and classified according to type of toxin into (4A; 1B; 2A&C). This result nearly similar to that recorded by (Rosec, *et al.* 1997; Abdaslam, *et al.* 2014; Ezzat, *et al.* 2014 and Abd El-Tawab, *et al.* 2015) who found enterotoxin A; C and A&C in beef meat and meat products.

The results obtained in Table (8) revealed that, 11 isolates of *Ps. aeruginosa* were isolated from examined frozen sausage samples represented as 2(5.7%) from samples of Benha city; 3(8.6%) from samples of centers and 6(17.1%) from samples of villages. These results came in accordance with those obtained by El-Shopary (2010); Nadim-Samaa (2012) and Hassan (2013). *A. hydrophila* strains were the only species isolated from examined samples. Only three frozen sausage samples collected from different villages were positive for *A. hydrophila* isolation, meanwhile, they failed to be detected in all examined samples of frozen sausage collected from Benha city and different centers at Kaliobia Governorate. Nearly similar results were obtained by Sharma and Kumar (2011) and Osman, *et al.* (2012) who isolated *A. hydrophila* strains from frozen meat samples.

Only two frozen sausage samples collected from different villages were positive for *Salmonella* isolation, meanwhile, they failed to be detected in all examined samples of frozen sausage collected from Benha city and different centers at Kaliobia Governorate. These results were agreed with those recorded by Fathi and Thabet (2001); Eleiwa (2003); El-Mossalami (2003); Zaki-Eman (2003); Abou Hussein-Reham (2004); Siriken, *et al.* (2006); Gibriel, *et al.* (2007); Abd El- Salam-Aza (2014); Abdel-Raouf, *et al.* (2014); Abd El-Tawab, *et al.* (2015) and Heweidy (2017) who failed to isolate *Salmonella* species from sausage samples or isolated them with lower percentages. Meanwhile, disagreed with those of Maarouf and Nassif-Marionette (2008); Al-Mutairi (2011); Radwan-Nermin (2013); Mousa, *et al.* (2014) and Djoulde, *et al.* (2015) who isolated *Salmonella* from frozen sausage samples with high incidence. Finally, the present study proved that frozen sausage has public health hazard and the presence of aerobic bacteria; Enterobacteriaceae; coliforms; *E.coli*; Staphylococci mainly Coagulase Positive *S. aureus*; *Ps. aeruginosa*; *A. hydrophila*; *Salmonella* and Psychrotrophic bacteria may be due to mishandling and the negligence of hygienic aspects. Therefore, it was concluded that these pathogens are meat borne pathogens of public health important.

References

1. Abd El-Aziz. "Chemical and technological studies on the processing of beef burger. M.Sc. Thesis, Food Science and Technology Department." Faculty of Agriculture Menofia University Egypt.
2. Abd El-Aziz-Wafaa, *et al.* "Bacterial hazards associated with consumption of street vended meat products in Kalyobia governorate". *Benha Veterinary Medical Journal* 28.2 (2015): 58-66.

3. Abd El-Tawab AA, *et al.* "Bacteriological studies on some food borne bacteria isolated from chicken meat and meat products in Kaliobia Governorate" 29.2 (2015): 47-59.
4. Abdaslam SA, *et al.* "Isolation of *Escherichia coli* O157 and other food borne pathogens from meat products and their susceptibility to different antimicrobial agents". *Current Research in Microbiology and Biotechnology* 2.3 (2014): 391-397.
5. Abdel Raouf M., *et al.* "Antimicrobial Activities of Some Herbs Extracts on Food Borne Bacteria". *Journal of American Science* 10.11 (2014): 76-85.
6. Abou Hussein Reham AA. "Microbial Evaluation of Some Meat Products". M. V. Sci. Thesis (Meat Hygiene Faculty of Veterinary Medicine Zagazig University. (Benha Branch).
7. Abou-Aly HE., *et al.* "Microbial and chemical quality of retailed sausage and antimicrobial effect of essential oils or lactic acid Bacteria against foodborne pathogens". 12th Conference of Microbiology, Cairo, Egypt: (2007): 1-15.
8. Adesiji YO, *et al.* "Prevalence of *Acrobacter*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria". *Sierra Leone Journal of Biomedical Research* 3: 8-12 (2011): 2076-6270.
9. Ahmed-Alyaa, S.O.S. 2015. Quality of Native and Imported Meat in The Egyptians Markets .M.Sc. Thesis (Food Hygiene and Control) Fac. Vet. Med. Cairo University.
10. Akhtar S., *et al.* "Microbiological food safety: a dilemma of developing societies". *Critical Reviews in Microbiology* 40.4 (2014): 348-359.
11. Mohammed Fatem Al-Mutairi. "The Incidence of Enterobacteriaceae Causing food Poisoning in some meat products". *Advance Journal of Food Science and Technology* 3.2 (2011): 116-121.
12. APHA. "Compendium of Methods for the Microbiological examination of Foods". 4th Ed. F.P. American Public Health Association (2001).
13. Argudin MA., *et al.* "Food poisoning and staphylococcus aureus enterotoxins". *Toxins* 2.7 (2010): 1751-1773.
14. Armany AG., *et al.* "Detection of some foodborne pathogens in meat products by Polymerase Chain Reaction". *Benha Veterinary Medical Journal* 30.1 (2016): 323-330.
15. Ayçiçek H., *et al.* "Assessment of the bacterial contamination on hands of hospital food handlers". *Food Control* 15.4 (2004): 253-259.
16. Bezerra AC., *et al.* "Microbiological quality of hamburgers sold in the streets of Cuiaba' – MT, Brazil and Vendor hygiene-awareness". *Food Science and Technology* 30.2 (2010): 520-524.
17. Cencil GB T, *et al.* "2003. Enterotoxin production by *Staphylococcus aureus* isolated from mastitic cows". *Journal of Food Protection* 66.9 (2003): 1693-1696.
18. Clarence SY, *et al.* "Assessment of Bacteriological quality of ready to eat food (Meat Pie) in Benin City metropolis, Nigeria". *African Journal of Microbiology Research* 3 .6 (2009): 390-395.
19. Djoulde, DR., *et al.* "Microbiological quality and safety of street meat-food sold in Soudano Sahelian zone of Cameroon". *International Journal of Current Microbiology and Applied Sciences* 4.2 (2015): 441-450.
20. Edward RP and Ewing WH. "Edwards and Ewing's identification of Enterobacteriaceae, 3rd Edition. Burgess, Minneapolis. (1986).
21. El- Mossalami E I K. Risk assessment of ready prepared meat products ph. D. Sc. Thesis, Fac. Vet. Med., Cairo Univ., Egypt. . (2003).
22. Eleiwa N Z H. "Effect of chemical preservatives on food poisoning bacteria in some locally manufactured meat products". Ph. D. Thesis (Meat hygiene), Fac. Vet. Med., Zagazig University, (Benha Br.) (2003).
23. El-Ghamry ST. "Incidence and public health importance of *Bacillus cereus* in meat and some meat products". M. V. Sc. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt. (2004).
24. El-Maghraby-Marwa SM. "Bacteriological evaluation of meat and some meat products at consumer level". M.V.Sc., Thesis (Meat Hygiene), Fac. Vet. Med., Sadat City. University (2014).
25. El-shopary NF. "Pseudomonas species in some retailed meat products. M. V. Sc. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt (2010).
26. Ercolini D, *et al.* "Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions". *Applied and Environmental Microbiology* 72.7 (2006): 4663-4671.

27. ES (Egyptian Standards) 2005. No1972/2005 on frozen sausage.
28. Ezzat M., *et al.* "Molecular characterization of pathogenic E.coli isolated from meat and their products". *Saudi Journal of Biological Sciences* 21.1 (2015): 103-113.
29. Farag HE., *et al.* "The incidence of proteolytic Pseudomonas species associated with ground beef with regards to their spoilage effect". *Assiut Veterinary Medical Journal* 119 (2008): 115-128.
30. Fathi SM and Thabet E R. "Incidence of Salmonella and E. Coli in packed meat products sold in Assiut city". *Assiut Veterinary Medical Journal* 46.91 (2001): 187 - 199.
31. FDA (Food and Drug Administration). Evaluation and definition of potentially hazardous foods. Analysis of microbial hazards related to time/temperature control of food for safety. Department of Health and Human Services. Food and Drug Administration Chapter 4 (2001):1-19.
32. Gergis M T. Some bacterial toxins in meat products. Ph. D. Sc. Thesis, Faculty of Veterinary Medicine Zagazig University Egypt. (2005).
33. Ghenghesh K S, "Aeromonas- Associated infections in developing countries". *The Journal of Infection in Developing Countries* 2.2 (2008): 81-98.
34. Gi Y L., *et al.* "Prevalence and classification of pathogenic E.coli isolated from fresh beef, poultry and pork in Korea". *International Journal of Food Microbiology* 134.3 (2009): 196-200.
35. Gibriel, AY. *et al.* "Application of Monascus purpureus pigments produced using some food industry wastes in beef sausage manufacture". *Egyptian Journal of Food Science* 35 (2007): 27-45.
36. Gwida M. *et al.* "Occurrence of Enterobacteriaceae in Raw Meat and in Human Samples from Egyptian Retail Sellers". *International Scholarly Research Notices* (2014): 1-6.
37. Hamed E A. *et al.* "Bacteriological hazard associated with meat and meat products". *Egyptian Journal of Agricultural Research* 93, 4 (B) (2015): 385-393.
38. Hamouda NM. "Microbiological risk assessment of some meat products". Ph. D. V. Sc. Thesis, Faculty of Veterinary Medicine, Suez Canal University, Egypt (2005).
39. Hassan M A. "Bacterial associated with fungal contamination in frozen beef meat. Thesis of master in microbiology". *Faculty of Veterinary Medicine, Alexandria University* (2013).
40. Hassanin Fatin S. "Bacterial Hazards Associated with Consumption of Some Meat products". *Benha Veterinary Medical Journal* 15.2 (2004):129-141.
41. Heweidy AYA. Prevalence of some foodborne Micro-organisms in meat and meat products. M V Sc. Thesis (Meat Hygiene), Faculty of Veterinary Medicine - Benha University (2017).
42. Hillers VN., *et al.* "Consumer food-handling behaviors associated with prevention of 13 foodborne illnesses". *Journal of Food Protection* 66.10 (2003):1893-1899.
43. Ibrahim Eman FM. Bacteriological evaluation of some street vended meat products M V Sc. Thesis, Meat hygiene, Faculty of Veterinary Medicine - Menoufia University (Sadat Branch). Egypt. (2008).
44. ICMSF (International commission of Microbiological Specification for Foods) 1996. Microorganisms in Food. Their Significance and Methods of Enumeration. 3rd Ed. University of Toronto, Canada.
45. Igarashi H., *et al.* "Latex agglutination test for Staphylococcus toxic Shock syndrome toxin1" *Journal of Clinical Microbiology* 23.3 (1986): 516-521.
46. ISO (International Standardization Organization). Microbiology of Food and Animal Feeding Stuff_Horizontal Method for Detection of Escherichia coli O157. ISO 1664. (2001).
47. ISO 6579 (International Standardization Organization) "Microbiology of food and animal feeding stuffs- horizontal method for the detection of Salmonella spp". *International standard*. (4th edition). (2002).
48. Kalantari S., *et al.* "Determination of bacterial contamination isolated from sandwiches in Kerman City and their resistance to commonly used antimicrobials". *Archives of Applied Science Research* 4.2 (2012): 1100-1105.

49. Kaper JB., *et al.* "Pathogenic Escherichia coli". *Nature Reviews Microbiology* 2.2 (2004): 123-140.
50. Karaboz I and Dincer B. "Microbiological investigation on some of the commercial frozen meat in IZMIR". *Turkish Electronic Journal of Biotechnology Special issue* (2002): 18-23.
51. Konemann E., *et al.* "Color Atlas and Textbook of Diagnostic Microbiology". *Fifth Edition*. Lippincott, Philadelphia, New York. (1997).
52. Lin K W and Huang C Y. "Physicochemical and textural properties of ultrasound – degraded konjac flour and their influences on the quality of low – fat Chinese – style sausage". *Meat Science* 79.4 (2008): 615-622.
53. Llewelyn M and Cohen J. "Superantigens: Microbial agents that corrupt immunity". *The Lancet Infectious Diseases* 2.3 (2002): 156-162.
54. Maarouf AA and Nassif Marionette Z. "Bacteriological studies on frozen cow meat and some meat products at Benha city". *Journal of the Egyptian Veterinary Medical Association* 68.1 (2008): 129-141.
55. Mahmoud Y El A. "Effect of thawing processes on microbial count of frozen beef". *Benha Veterinary Medical Journal* 1:34-43 (2001).
56. Mansour Amal FA. "Occurrence of Escherichia coli in some beef products at Alexandria markets". *Animal Health Research journal* 1.2 (2013): 50-54.
57. Mantis FN., *et al.* "Safety and shelf-life of widely distributed vacuum packed heat treated sausages". *Bulgarian Journal of Veterinary Medicine* 8.4 (2006): 245-254.
58. Markey BK., *et al.* "Clinical Veterinary Microbiology 2nd edition. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto (2013).
59. Mosupye FM and Van Holy A. "Microbiological hazard identification and exposure assessment of street food vending in Johannesburg, South Africa". *International Journal of Food Microbiology* 61.23 (2000): 137-145.
60. Mousa MM., *et al.* "Microbiological Criteria of Some Meat Products". *Alexandria Journal of Veterinary Sciences* 42.1(2014): 83-89.
61. Nadim Samaa SA. "Prevalence of Enteropathogenes in frozen meat and chicken with special reference to *E.coli* and *Salmonellae*". M.V c Thesis (Food Hygiene and control) (2012).
62. Nicky BB. Bacteria from fish and other aquatic animals (a practical identification manual).CABI publishing is a division of CAB international. QR106.B85 P (83-116) (2004).
63. Oluwafemi F and Simisaye M T. "Extent of microbial contamination of sausages sold in tow Nigerian cities". *African Journal of Biomedical Research* 9 (2006): 133-139.
64. Osman K., *et al.* "Molecular detection of the Aeromonas virulence aerolysin gene in retail meats from different animal sources in Egypt". *World Journal of Microbiology and Biotechnology* 28.5 (2012): 1863-1870.
65. Oterize JM., *et al.* 2006. "Isolation and sausage (morcillas)". *Journal of Food Microbiology* 23 (2006): 283-288.
66. Ouf Jehan M. "Microorganisms of sanitary importance in some meat products additives". Ph D. Thesis (meat hygiene), Faculty of Veterinary Medicine, Cairo University, Egypt (2001).
67. Plaatjies Z. "Staphylococcal growth in fresh vacuum-packed red meat at various storage conditions". 8th World Congress on Environmental Health. Durban, South Africa (2004).
68. Protocarrero S., *et al.* "Staphylococcus aureus survival, Staphylococcal enterotoxin Production and shelf stability of country cured hams manufactured under different processing procedures". *Meat Science* 62.21 (2002): 267-273.
69. Quinn PJ., *et al.* "Veterinary microbiology and microbial disease". *Iowa State University Press Blackwell Science Ltd, chapters* (2002): 84-96.
70. Ramadan AM. "Contamination of meat products with human pathogens". M.V. Sc. Thesis (Meat hygiene), Faculty of Veterinary Medicine, Alexandria University (2015).
71. Rosec JP., *et al.* "Enterotoxin production by Staphylococci isolated from foods in France". *International Journal of Food Microbiology* 35.3 (1997): 213-221.
72. Sachindra NM., *et al.* "Microbial profile of buffalo sausage during processing and storage". *Food Control* 16.1 (2005): 31-35.

73. Sharma I and Kumar A. "Occurrence of enterotoxigenic *Aeromonas* species in food of animal origin in North East India". *European Review for Medical and Pharmacological Sciences* 15.8 (2001): 883-887.
74. Sharoba AM. "Quality attributes of sausage substituted by different levels of whole amaranth meal." *Annals of Agricultural Science Moshtohor* 47 (2009) 105 -120.
75. Shawish RR. "Prevalence of shiga toxin-producing *Escherichia coli* in some beef products". Ph.D. Thesis (Meat Hygiene). Faculty of Veterinary Medicine - Menoufia University (Sadat branch). (2015).
76. Siriken B., *et al.* "A note on the incidences of *Salmonella* spp., *Listeria* spp. and *Escherichia coli* O157:H7 serotypes in Turkish sausage (Soudjouck)". *Meat Science* 73.1 (2006):177-181.
77. Snedecor GW and Cochran WG. "Statistical Methods, 6th Ed. Iowa State University Press". *Asmes*, USA (1969).
78. Sobieh A S A. "Fast meat meals at restaurant level in Cairo Governorate". M. V. Sc. Thesis, meat hygiene, Faculty of Veterinary Medicine - Benha University (2014).
79. Soultos N., *et al.* "Incidence of some food Greece". *Archis. Furet. Lebensmittel. Hygiene*, 54.3 (2003): 55-57.
80. SPSS for windows, Version: 11 (19 September 2001). Copyright SPSS Inc. 1989 - 2001. All rights reserved.
81. Stagnitta PV., *et al.* "Prevalence of some bacteria yeasts and molds in meat foods in san Luis, Argentina". *Central European Journal of Public Health* 14 .3 (2006): 141-144.
82. Tarabees RZ., *et al.* "Polymerase Chain Reaction (PCR): An Alternative Rapid Method for Detection of Some Microbial Contamination of Meat Products". *Alexandria Journal of Veterinary Sciences* 45.1 (2015): 91-98.
83. Todar's on line text book of bacteriology. Text book of bacteriology .net (2004_06_04) Retrieved on 2011_10_09.
84. Woody JM., *et al.* "Comparison of the Difco *E.coli* rapid detection system and petrifilm test kit-HEC for detection of *Escherichia coli* O157: H7 in fresh and frozen ground beef". *Journal of Food Protection* 61.1 (1998): 110-112.
85. Zaki Eman M. "Risk assessment of ready prepared meat products". Ph. D. Thesis, (Meat Hygiene). Faculty of Veterinary Medicine, Cairo University (2003).
86. Zweifel C., *et al.* "Microbiological contamination of cattle and pig ' carcasses at five abattoirs determined by swab sampling in accordance with Eu. Decision 2001/471/EC". *Meat Science* 69.3 (2005): 559-566.

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