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 $5.88 \times 10^4 \pm 0.17 \times 10^4$; $2.98 \times 104 \pm 0.11 \times 10^4$; $2.36 \times 10^2 \pm 0.11 \times 10^2$; $1.93 \times 10^2 \pm 0.10 \times 10^2$ 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*

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

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 hygienicmeasures during meat production and bad storage conditions for frozen meat products can be measured using the aerobic plate count, total psychotroph 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- immerging 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	$3.62 \times 10^4 \pm 0.19 \times 104^c$
Centers	0	0.0	35	100.0	3.8×10^4	7.5×10^4	$5.88 \times 10^4 \pm 0.17 \times 104^b$
Villages	0	0.0	35	100.0	4.8×10^4	9.9×10^4	$7.91 \times 10^4 \pm 0.16 \times 104^a$

*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 ± SEM**
	No.	%*	No.	%*			
Benha city	4	11.4	31	88.6	0.7×104	$3.4×10^4$	$2.10 × 10^4 ± 0.13 × 10^{4c}$
Centers	3	8.6	32	91.4	1.9×104	$4.4×10^4$	$2.98 × 10^4 ± 0.11× 10^{4b}$
Villages	0	0.0	35	100.0	3. 2×104	$8.0×10^4$	$5.32 × 10^4 ± 0.22 × 10^{4a}$

*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 ± SEM**
	No.	%*	No.	%*			
Benha city	11	31.4	24	68.6	$0.8 × 10^2$	$2.7 × 10^2$	$1.53 × 10^2 ± 0.09 ×10^{2c}$
Centers	7	20.0	28	80.0	$1.6 × 10^2$	$3.8 × 10^2$	$2.36 × 10^2 ± 0.11×10^{2b}$
Villages	2	5.7	33	94.3	$1.9 × 10^2$	$6.7 × 10^2$	$4.84 × 10^2 ± 0.24×10^{2a}$

*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 ($P ≤ 0.05$) 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 ±SEM**
	No.	%*	No.	%*			
Benha city	23	65.7	12	34.3	$0.4 × 10^2$	$2.1 × 10^2$	$0.92 ×10^2 ± 0.13 × 10^{2c}$
Centers	12	34.3	23	65.7	$1.1 × 10^2$	$2.9 × 10^2$	$1.93 × 10^2 ± 0.10 × 10^{2b}$
Villages	4	11.4	31	88.6	$2.4 × 10^2$	$6.5 × 10^2$	$4.33 ×10^2 ± 0.22 × 10^{2a}$

*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 accept-ed 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
E.coli serotype	No.	%*	No.	%*	No.	%*	
O55:K59(B5)	1	2.86	2	5.71	3	8.57	EPEC
0125:K59(B5)	1	2.86	1	2.86	3	8.57	ETEC(EPEC)
0111:K58(B9)	0	0.0	1	2.86	1	2.86	EHEC
0126: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*

ETEC: 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 ±SEM**
	No.	%*	No.	%*			
Benha city	9	25.7	26	74.3	0.8×10^2	2.6×102	$1.62 \times 10^2 \pm 0.10 \times 10^{2c}$
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 Aeromoneus 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 $3.62 \times 10^4 \pm 0.19 \times 104$; $5.88 \times 10^4 \pm 0.17 \times 10^4$ 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 Heweidy (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 4.4×104 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 of 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 of were agree 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 0.4×102 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 O55:K59 (B5); one 0125:K59 (B5) and one 0126:K71 (B16); 5 (14.3%) from samples of centers with serotypes 2 O55: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 O111:K58(B9) and one O126: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 electrics 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.* 2011and 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 Plaatjies., *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.

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