Prevalence of some foodborne microorganisms in meat and meat products.

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A B S T R A C T

This study was conducted on 140 random samples of fresh beef and meat products viz: minced meat, luncheon and sausage (35 for each), collected from different shops at El-Kaliobia Governorate, to evaluate their bacteriological profile. The bacteriological examination of fresh beef and meat products minced meat, sausage and luncheon revealed that the mean values of APC, Enterobacteriaceae, coliform and Staphylococcus counts were $8.34\times10^4\pm0.10\times10^4$; $2.14\times10^2\pm0.97\times10^2$; $1.25\times10^2\pm0.13\times10^2$ and $2.36\times10^2\pm0.12\times10^2$ for fresh beef samples; $8.03\times10^4\pm0.12\times10^4$; $2.02\times10^2\pm0.76\times10^2$; $0.89\times10^2\pm0.06\times10^2$ and $2.67\times10^2\pm0.11\times10^2$ for minced meat samples; $6.74\times10^4\pm0.28\times10^4$; $1.85\times10^2\pm0.64\times10^2$; $0.73\times10^2\pm0.08\times10^2$ and $1.9\times10^2\pm0.11\times10^2$ for sausage samples and $5.85\times10^4\pm0.24\times10^4$; $1.69\times10^2\pm0.70\times10^2$; $0.71\times10^2\pm0.07\times10^2$ and $1.68\times10^2\pm0.11\times10^2$, for luncheon samples. Further, 21 isolates of *E.coli* were isolated from examined meat samples represented as 5(14.3%) from fresh beef with serotypes 2 O55:H7,1 O125:H18,1 O111: H4 and 1 O26:H11 ; 8(22.9%) from minced meat with serotypes 3 O55:H7, 2 O125:H18, 1 O111:H4, 1 O26:H11 and 1 untyped; 6 (17.1%) from sausage with serotypes 2 O55:H7, 1 O125:H18, 1 O111:H4 , 1 O26:H11 and one untyped and 2 (5.7%) from luncheon samples with serotypes O55:H7 . In addition, 25 isolates of Coagulase positive *S. aureus* were isolated from examined meat samples represented as 6 (17.1%) from fresh beef; 9 (25.7%) from minced meat; 7 (20.0%) from sausage and 3(8.6%) from luncheon samples. Meanwhile, Salmonella serovars were not detected from all examined meat samples.

Key words: Meat products, bacteriological evaluation, *E. coli*, *Staph. aureus*, Salmonella

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1. INTRODUCTION

Food borne diseases caused mainly by *E. coli*, Salmonella species and *S. aureus* are the major causes of mortality and infections especially in the developing countries. These pathogens are transmitted mainly through consumption of contaminated food and the presence of these organisms in meat and raw meat products has relevant public health implications (Zafar et al., 2016). The bacterial contamination and hygienic measures during meat production can be measured using the aerobic plate count, total Enterobacteriaceae, total coliforms and *Escherichia coli*. (Aberle et al., 2000; Hamed et al., 2015; McEvovy et al., 2004). *E. coli* is considered as a good indicator of possible fecal contamination (Synge, 2000). It is commonly non-virulent but some strains have adopted pathogenic or toxigenic virulence factors that make them pathogenic to human and animals. It has been associated with numerous out breaks of disease resulting from contaminated beef and meat products, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia (Datta et al., 2012; Gi et al., 2009; Kaper et al., 2004 ; Madden et al., 2001). Infections with Salmonellae and coagulase positive *S. aureus*, are the causative agents of two thirds of food-borne disease outbreaks causing gastroenteritis and rarely acquired directly from raw meat but mostly occurs either due to excessive handling or contamination during or after cooking of meat and meat products (Busani et al., 2005; Khan et al., 2014; Leloir et al., 2003). Staphylococci produce some enzymes which are implicated with Staphylococcus invasiveness and many extracellular substances some of which are heat stable enterotoxins that renders the food dangerous even though it appears normal and extensive cooking can be killed the bacteria but the toxins may not be destroyed because most of them are gene based i.e. they can be carried on the plasmid (Prescott et al., 2005). The enterotoxins are produced by about one-third of coagulase positive *Staph. aureus* strains and growth of enterotoxigenic strains of *Staph. aureus* to population of at least 10^5 cfu/g in food is generally considered necessary for production of sufficient amount of enterotoxins to induce food intoxication (Park et al., 1994). The Staphylococcal enterotoxins (SEs) are responsible for the symptoms. The disease is characterized by symptoms including nausea, vomiting, abdominal cramps and diarrhea lasting from 24 to 48 h and the complete recovery usually occurs within 1-3 days (Llewelyn and Cohen, 2002). Moreover, SEA is the most common enterotoxin recovered from food poisoning outbreaks (Balaban and Rasooly, 2000). As the level of contamination of beef and its products with different food-borne pathogens constitutes serious problems for consumers, so, the present study was conducted to evaluate the safety and quality of beef and common meat products (minced meat; sausage and luncheon) at Kaliobia Governorate.

2. MATERIAL AND METHODS

2.1. Samples collection:

A total of 140 random samples of fresh beef and meat products viz: Minced meat, luncheon and sausage (35 for each), were collected from different shops at Kaliobia Governorate. To evaluate the bacterial status and detection of some food borne pathogens containing them.

2.2. Bacteriological examination

1. Preparation of samples (American Public Health Association "APHA", 1992).
2. Determination of Aerobic Plate Count (APC)/ g, using the standard plate count following (Food and Drug Administration "FDA", 2001).
3. Determination of Total Enterobacteriaceae count by the surface plating method of ICMSF (1996) using Violet Red Bile Glucose agar medium (VRBG).
4. Total coliform count by the surface plating method of ICMSF (1996) using Violet Red Bile agar medium.
5. Isolation and identification of *E.coli* followingInternational Organization of Standardization "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 "SEIKEN" 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).
6. Determination of Total Staphylococci count following ICMSF (1996)
7. Isolation of *Staph. 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)
8. Isolation and identification of Salmonella following ISO (2002). Suspected Salmonella colonies that appeared as red with black centers on XLD agar were identified morphologically by Gram-stain and biochemically according to Quinn et al. (2002).

2.3. Statistical analysis:

Data obtained were analyzed according to Snedecor and Cochran (1969) using the computer software program (SPSS, 2001).

3. RESULTS

The results of bacteriological examination of fresh beef and meat products revealed that APC, coliform and enterobacteriaceae were highest in fresh beef then minced meat then sausage then luncheon. While, staphylococcal count was highest in minced meat then fresh beef then sausage then luncheon, in which the incidence of coagulase positive *Staph. aureus* is highest in minced meat then sausage then fresh beef then luncheon. Isolation and identification of *E. coli* in the examined samples revealed that the incidence of *E. coli* was highest in minced meat followed by sausage then fresh beef then luncheon, strains of *E.* coli identified as O_{55}:H_7, O_{125}:H_{18}, O_{111}:H_4, O_{26}:H_{11}, noticed that two samples were untypable. Salmonella serovars were failed to be detected in all examined samples of fresh beef and meat products.

4. DISCUSSION

Food borne diseases caused by *E. coli*, Salmonella species and *Staph. aureus* that transmitted mainly through consumption of contaminated food and the presence of them in meat and raw meat products has relevant public health implications

Table (1): Aerobic plate counts /g. (APC) in the examined samples of fresh beef and meat products (n=35 for each product)

Sample	Positive No.	%*	Min.	Max.	Mean ±SEM**
Fresh beef	35	100	7.3×10^4	9.9×10^4	$8.34\times10^4\pm0.10\times10^{4a}$
Minced meat	35	100	6.5×10^4	9.5×10^4	$8.03\times10^4\pm0.12\times10^{4a}$
Sausage	35	100	2.8×10^4	8.9×10^4	$6.74\times10^4\pm0.28\times10^{4b}$
Luncheon	35	100	2.5×10^4	7.9×10^4	$5.85\times10^4\pm0.24\times10^{4c}$

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

Table (2): Enterobacteriaceae counts/g. in the examined samples of fresh beef and meat products (n=35 for each product)

Sample	Positive No.	%*	Min.	Max.	Mean ±SEM**
Fresh beef	34	97.1	1.2×10^2	3.3×10^2	$2.14\times10^2\pm0.97\times10^{2a}$
Minced meat	34	97.1	1.1×10^2	2.9×10^2	$2.02\times10^2\pm0.76\times10^{2ab}$
Sausage	34	97.1	1.2×10^2	2.7×10^2	$1.85\times10^2\pm0.64\times10^{2bc}$
Luncheon	34	97.1	1.0×10^2	2.6×10^2	$1.69\times10^2\pm0.70\times10^{2c}$

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

Table (3): Coliforms counts/g. in the examined samples of fresh beef and meat products (n=35 for each product)

Sample	Positive No.	%*	Min.	Max.	Mean ±SEM**
Fresh beef	14	40.0	0.6×10^2	2.1×10^2	$1.25\times10^2\pm0.13\times10^{2a}$
Minced meat	15	42.9	0.5×10^2	1.3×10^2	$0.89\times10^2\pm0.06\times10^{2b}$
Sausage	13	37.1	0.3×10^2	1.1×10^2	$0.73\times10^2\pm0.08\times10^{2b}$
Luncheon	13	37.1	0.3×10^2	1.2×10^2	$0.71\times10^2\pm0.07\times10^{2b}$

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

Table (4): Incidence of *E. coli* in examined samples of fresh beef and meat products (n=35 for each product)

Sample	Positive No.	%*	No. of accepted samples**	No. of non- accepted samples**
Fresh beef	5	14.3	30	5
Minced meat	8	22.9	27	8
Sausage	6	17.1	29	6
Luncheon	2	5.7	33	2
Total	21	15.0	119	21

* Percentage in relation to total number of sample in each row. **Accepted and non- accepted samples according to (EEC, 2005).

Table (5): Incidence and serotyping of *E. coli* isolated from positive samples of fresh beef and meat products (n=35 for each product)

Sample	Fresh beef		Minced meat		Sausage		Luncheon		Strain
E. coli serotype	No.	%*	No.	%*	No.	%*	No.	%*	characteristic
O55:H7	2	5.71	3	8.57	2	5.71	2	5.71	EPEC
O125:H18	1	2.86	2	5.71	1	2.86	0	0.00	EPEC
O111:H4	1	2.86	1	2.86	1	2.86	0	0.00	EHEC
O26:H11	1	2.86	1	2.86	1	2.86	0	0.00	EPEC
Untyped	0	0.00	1	2.86	1	2.86	0	0.00	-
Total	5	14.29	8	22.86	6	17.14	2	5.71	-

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

Table (6): Staphylococci counts/gm. in the examined samples of fresh beef and meat products (n=35 for each product)

Sample	Positive		Min.	Max.	Mean ±SEM**
	No.	%*			
Fresh beef	34	97.1	0.7×10^{2}	3.8×10^{2}	$2.36\times10^{2}\pm0.12\times10^{2a}$
Minced meat	34	97.1	1.1×10^{2}	3.8×10^{2}	$2.67\times10^{2}\pm0.11\times10^{2a}$
Sausage	33	94.3	0.7×10^{2}	3.3×10^{2}	$1.9\times10^{2}\pm0.11\times10^{2b}$
Luncheon	33	94.3	0.7×10^{2}	2.7×10^{2}	$1.68\times10^{2}\pm0.11\times10^{2b}$

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

Table (7): Incidence of Coagulase Positive *S. aureus* in examined samples of fresh beef and meat products (n=35 for each product)

Sample	Positive		No. of accepted samples**	No. of non- accepted samples**
	No.	%*		
Fresh beef	6	17.1	29	6
Minced meat	9	25.7	26	9
Sausage	7	20.0	28	7
Luncheon	3	8.6	32	3
Total	25	17.9	115	25

* Percentage in relation to total number of sample in each row. **Accepted and non- accepted samples according to (EEC, 2005).

(Normanno et al., 2007). The total aerobic and Enterobacteriaceae counts reflect the bacterial contamination and declared the hygienic quality of both raw meat and meat products. Meanwhile, Coliform counts may indicate faecal contamination either from human or animal sources and its presence indicate poor sanitation and handling (McEvovy et al., 2004; Paulsen et al., 2006).

The data shown in Table (1) revealed that the minimum and the maximum aerobic plate counts (APC) in the examined of fresh beef and meat products (minced meat, sausage and luncheon) were ranged from 7.3×10^{4} to 9.9×10^{4}; 6.5×10^{4} to 9.5×10^{4}; 2.8×10^{4} to 8.9×10^{4} and 2.5×10^{4} to 7.9×10^{4} respectively, with a mean value of $8.34\times10^{4}\pm0.10\times10^{4}$; $8.03\times10^{4}\pm0.12\times10^{4}$; $6.74\times10^{4}\pm0.28\times10^{4}$ and $5.85\times10^{4}\pm0.24\times10^{4}$, respectively. All examined meat samples (100%) contained microorganisms. However, the counts were considered satisfactory as these results were lower than those suggested by EEC (2005). Nearly similar counts were recorded by Ahmed-Alyaa (2015); Hamed et al. (2015); Hassan and Soultan (2004); Paulsen et al. (2006).

The results in Table (2) appeared that, the minimum and the maximum Enterobacteriaceae count in the examined fresh beef and meat products(minced meat ,sausage and luncheon)were ranged from 1.2×10^{2} to 3.3×10^{2}; 1.1×10^{2} to 2.9×10^{2}; 1.2×10^{2} to 2.7×10^{2} and 1.0×10^{2} to 2.6×10^{2}, respectively, with a mean value of $2.14\times10^{2}\pm0.97\times10^{2}$; $2.02\times10^{2}\pm0.76\times10^{2}$; $1.85\times10^{2}\pm0.64\times10^{2}$ and $1.69\times10^{2}\pm0.70\times10^{2}$, respectively. These results of were agree with those of Hassan and Soultan (2004); Stagnitta et al. (2006) and Gwida et al. (2014).

Data presented in Table (3) showed that, the minimum and the maximum Coliform count in the examined fresh beef and meat products (minced meat, sausage and luncheon) were ranged from 0.6×10^{2} to 2.1×10^{2}; 0.5×10^{2} to 1.3×10^{2}; 0.3×10^{2} to 1.1×10^{2} and 0.3×10^{2} to 1.2×10^{2}, respectively, with a mean value of $1.25\times10^{2}\pm0.13\times10^{2}$; $0.89\times10^{2}\pm0.06\times10^{2}$; $0.73\times10^{2}\pm0.08\times10^{2}$ and $0.71\times10^{2}\pm0.07\times10^{2}$, respectively. These results came in parallel with those of Paulsen et al. (2006) Stagnitta et al. (2006); Ahmed-Alyaa (2015); Al-Mutairi (2011) and Hamed et al. (2015).

The results in Tables (4&5) revealed that, 21 isolates of *E.coli* were isolated from examined meat samples represented as 5(14.3%) from fresh beef with serotypes 2 O55:H7,1 O125:H18,1 O111: H4 and 1 O26:H11 ; 8(22.9%) from minced meat with serotypes 3 O55:H7, 2 O125:H18, 1 O111:H4, 1 O26:H11 and 1 untyped; 6 (17.1%) from sausage with serotypes 2 O55:H7, 1 O125:H18, 1 O111:H4 , 1 O26:H11 and one untyped and two(5.7%) from luncheon samples with serotypes O55:H7 . Moreover, 119 samples out of 140 ones were accepted, as they were free from *E. coli* isolates according to (EEC, 2005). Nearly similar results were obtained by Abd El-Salam-Azza (2014); Abdaslam et al. (2014); Maarouf and Nassif-Marionette (2008); Ramadan (2015); Tarabees et al. (2015). Meanwhile, these results were disagreed with those of EI Jakee et al. (2014); Elnawawi et al. (2012); Gwida et al. (2014); Nychas et al. (2008) and Kamal-Asmaa (2015). Moreover, the same serotypes of *E.coli* were previously isolated by Abd El-Salam-Azza (2014); Kalchayanand et al. (2012); Mohammed et al. (2014); Windham et al. (2013) and EI Jakee et al. (2014).

The obtained results in Table (6) revealed that, the minimum and the maximum Staphylococcus count in the examined fresh beef and meat products(minced meat ,sausage and luncheon) were ranged from 0.7×10^2 to 3.8×10^2; 1.1×10^2 to 3.8×10^2; 0.7×10^2 to 3.3×10^2 and 0.7×10^2 to2.7×10^2, respectively, with a mean value of $2.36\times10^2\pm0.12\times10^2$; $2.67\times10^2\pm0.11\times10^2$; $1.9\times10^2\pm0.11\times10^2$ and $1.68\times10^2\pm0.11\times10^2$, respectively. These counts came in agreement with Phillips et al. (2006); Plaatjies et al. (2004) and Ahmed-Alyaa (2015).

The results obtained in Table (7) revealed that, 25 isolates of Coagulase Positive *Staph. aureus* was isolated from examined meat samples represented as 6 (17.1%) from fresh beef; 9 (25.7%) from minced meat; 7 (20.0%) from sausage and 3(8.6%) from luncheon samples. Moreover, 115 samples out of 140 ones were accepted, as they were free from Coagulase Positive *Staph. aureus* isolates according to (EEC, 2005). These results came in accordance with those obtained by Goja et al. (2013); Kanika et al. (2011); Maarouf and Nassif-Marionette (2008) and Hamed et al. (2015). Meanwhile, these results were disagreed with those of Abd El-Hady (2015); Abdaslam et al. (2014); EI Jakee et al. (2014) and Tarabees et al. (2015) who isolated *S. aureus* from raw meat and meat products with high incidence.

The present study failed to detect Salmonella serovars from all examined meat samples. These results were agreed with those recorded by Datta et al. (2012) and Abdel-Raouf et al. (2014)et al., (2014). Meanwhile, disagreed with those of Abdaslam et al. (2014); Maarouf and Nassif-Marionette (2008); Ramadan (2015) who isolated Salmonella from beef and meat products.

Finally, the present study proved that beef and meat products are considered public health hazard and the presence of aerobic bacteria; Enterobacteriaceae; coliforms; *E. coli* and Staphylococci Mainly Coagulase Positive *Staph. aureus* may be due to mishandling and the negligence of hygienic aspects either at production levels where most workers did not have medical certificates or selling of meat with expired dates. Therefore, it was concluded that *E. coli* and coagulase positive *Staph. aureus* are meat-borne pathogens of public health important.

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