



Bacterial evaluation of vacuum packaged meat products.

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ABSTRACT

The purpose of this study to evaluate the microbiological quality of vacuum packaged meat product samples (luncheon-sausage) collected from different markets in Qalyobia governorate (30 of each). The obtained results indicated that the mean values of APC, anaerobic plate count and Enterobacteriaceae counts was $2.1 \times 10^6 \pm 1.5 \times 10^6$, $1.5 \times 10^7 \pm 3.5 \times 10^6$ & $1.7 \times 10^4 \pm 3.9 \times 10^3$ cfu/g for sausage and $2.9 \times 10^5 \pm 2.6 \times 10^4$, $2.4 \times 10^5 \pm 2.5 \times 10^4$ & $2 \times 10^5 \pm 2 \times 10^4$ cfu/g for luncheon, respectively. Isolation and identification of some food poisoning bacteria were carried out. Salmonella, Staphylococci, and *Clostridium perfringens* were isolated and identified and the incidence was 10 %,6.6% and 33.3 % for sausage and 3.3, 10%&43.3 % for luncheon, respectively. Contamination of food by handlers is the most common cause of the presence of microorganisms which indicate a bad hygienic measure applied through different stages of food preparation, handling and serving.

Key Words: vacuum, sausage, luncheon, salmonella

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1. INTRODUCTION

Meat products such as sausage, luncheon are gaining popularity because they represent quick easily prepared meat meals and solve the problem of the shortage in fresh meat of high price which is not within the income of large numbers of families. Microorganisms may contaminate meat products during a long chain of processing from the time of preparation, handling, processing, distribution and storage as well as marketing. Such contamination may render the products of inferior quality or even unfit for human consumption and at times may constitute a public health hazard. Possibility of contamination of meat products with food poisoning bacteria especially Salmonella organisms has been extensively reported (Reham, 2004). Salmonella are found worldwide and universally recognized as zoonotic agent. The primary habitat of salmonella is the intestinal tract of animals and humans. Additionally, salmonella cause illness by means of infection, as it multiplies in the intestine, colonizes and subsequently invades the intestinal tissue, producing an enterotoxin which causing inflammatory reaction and diarrhea (ICMSF, 2006). Also, *Clostridium perfringens* is one of the most widespread pathogenic bacteria in the environment and is commonly found (although

in low numbers) in the gastrointestinal tract of healthy animals, from where it generally contaminates animal carcass during slaughtering (Aberle *et al.*, 2001). Farther, staphylococcal food poisoning is one of major concern in public health programs worldwide. *Staphylococcus aureus* is a leading cause of gastroenteritis result from consumption of meat in which enterotoxigenic Staphylococci have grown and produced enterotoxins. Staphylococcal enterotoxins are considered potential biological threat because of their stability at high temperature (100°C for 1 hr.) and their ability to incapacitate individuals for several days to two weeks (Bhatia and Zahoor, 2007).

Therefore, bacterial evaluation of vacuum packaged meat products (luncheon –sausage) was the purpose of current study to detect aerobic and anaerobic as well as enteric bacteria.

2. MATERIAL AND METHODS

2.1. Collecting of samples:

A grand Total of 60 random samples of sausages and luncheon (30 of each) were collected from different markets in Qalyobia governorate.

The collected samples were kept in separate sterile plastic bags and transferred directly to the laboratory of bacteriology in an ice box under complete aseptic conditions without delay, to be bacteriology examined.

2.2. Preparation of samples: (APHA, 2001)

25 grams of each sample were weighed and transferred into a sterile homogenizer flask containing 225 ml of 0.1% sterile buffered peptone water then homogenized at 2000 rpm for 1-2 min. to provide a homogenate of 1/10 dilution, one ml from the original dilution was transferred to another sterile tube containing 9 ml of 0.1% sterile buffered peptone water and mixed well to make the next dilution, from which further decimal serial dilutions were prepared.

2.2.1. Aerobic Plate Count (ICMSF, 1996)

One ml from each of the previously prepared serial dilutions was transferred into two separate sterile petri dishes, using pour plate method, to which approximately 15 ml of sterile melted and tempered plate count agar (45°C) were added. After thorough mixing, the incubated plates were allowed to solidify before being incubated at 27 °C for 24 hr. The aerobic plate count per gram calculated on plates containing 20-200 colonies was counted and each count was recorded separately.

2.2.2. Total Enterobacteriaceae Count (ISO, 2004)

From each of the previously prepared serial dilutions, 0.1 ml were poured into duplicate set of petri dishes, previously inoculated with 10 ml of sterile Violet Red Bile Glucose agar medium (VRBG). Then after evenly similar equal mixing, cover layer (tempered promptly to about 45 °c) of approximately 5 ml of (VRBG) agar was poured overall the plates. After thorough mixing the inoculated plates were allowed to solidify at room temperature before being inoculation. The plates were incubated at 27 °C for 24 hours. All purple suspected colonies surrounded by a purple halo were counted, the Enterobacteriaceae count / g was calculated.

2.2.2.1. Confirmatory test (ICMSF, 1996)

Two typical colonies were streaked into plates of Violet Red Bile Glucose agar and incubated at 25 - 27 °C for 20 - 24 hours, from which two colonies surrounded by purple precipitation zone were separately streaked onto slants of nutrient agar. The culture was inoculated into tubes of Glucose medium. The latter was covered with a sterile mineral oil then incubated at 27 °C for 20 - 24 hours. Oxidase test was carried out from the

belonging to Enterobacteriaceae when the oxidase test was negative, while the glucose broth was turned to yellow.

2.2.3. Anaerobic Bacterial Count (Roberts et al., 1995):

One ml from each of previously prepared serial dilution was spread into reinforced clostridial agar media. The plates were then incubated in upright position in anaerobic jar (Mackintosh jar) at 27 °C for 24hr. The suspected plates were selected and counted, and the results were interpreted as colony forming units (cfu) per gram of the samples.

3. RESULTS:

It is evident from the result recorded in table (1) that the APC of the examined samples of vacuum packaged meat products ranged from 1.1×10^3 to 4.5×10^7 with an average of $2.1 \times 10^6 \pm 1.5 \times 10^6$ for sausage and 1.1×10^5 to 6.2×10^5 with an average of $2.9 \times 10^5 \pm 2.6 \times 10^4$ for luncheon. Table (2) indicated that total anaerobic plate count of the examined samples of vacuum packaged meat products was 1.1×10^5 to 7.1×10^7 with an average of $1.5 \times 10^7 \pm 3.5 \times 10^6$ for sausage and 1.2×10^5 to 4.2×10^5 with an average of $2.4 \times 10^5 \pm 2.5 \times 10^4$ for luncheon.

Also, Table (3) revealed the total Enterobacteriaceae count of the examined samples of vacuum packaged meat products was 3.6×10^2 to 7.8×10^4 with an average $1.7 \times 10^4 \pm 3.9 \times 10^3$ for sausage and 1.1×10^5 to 3.5×10^5 with an average $2 \times 10^5 \pm 2 \times 10^4$ for luncheon samples. Tables (4) revealed that the incidence and serotyping of *Salmonella* isolated from the examined samples by 10% for sausage and 3.3% for luncheon which identified serologically as *S.typhimurium* (1%) and *S.entretidis* (2%) .

Table (5) referred to the *C.perfringens* isolated from the examined vacuum packaged meat samples with 33.3% for sausage and with 43.3% for luncheon. Moreover, Table (6) reported the incidence of Lecithinase +ve strains in the examined samples of sausage were 7 of 10 (70%) of *C. perfringens* and Lecithinase -ve strains were 3 of 10 (30%). Lecithinase +ve strains in the examined samples of luncheon were 10 of 13 (77%) and Lecithinase -ve strains were 3 of 13 (23%).

The results reported in table (7) showed that +ve lecithinase strains of *C.perfringens* isolates were typing into type A,B,C and D toxins. Type A appeared in 70% of +ve Lecithinase in sausage samples and 100% in luncheon samples and B, C,

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Table (1): Aerobic Plate Count (APC) of the examined vacuum packaged meat products samples (n=30 of each)

Samples	Positive samples		Minimum	Maximum	Mean ± SE
	NO.	%			
Sausage	30	100	1.1×10^3	4.5×10^7	$2.1 \times 10^5 \pm 1.5 \times 10^6$
Luncheon	30	100	1.1×10^5	6.2×10^5	$2.9 \times 10^5 \pm 2.6 \times 10^4$

S.E*=standard error of mean

Table (2): Anaerobic Plate count of the examined vacuum packaged meat products samples (n=30 of each).

Samples	Positive samples		Minimum	Maximum	Mean ± SE
	NO.	%			
Sausage	18	60	1.1×10^5	7.1×10^7	$1.5 \times 10^7 \pm 3.5 \times 10^6$
Luncheon	21	70	1.2×10^5	4.2×10^5	$2.4 \times 10^5 \pm 2.5 \times 10^4$

S.E*=standard error of mean

Table (3): Enterobacteriaceae Plate Count of the examined vacuum packaged meat products samples (n=30 of each).

samples	Positive samples		Minimum	Maximum	Mean ± SE
	NO.	%			
Sausage	22	73.3	3.6×10^2	7.8×10^4	$1.7 \times 10^4 \pm 3.9 \times 10^3$
Luncheon	20	66.6	1.1×10^5	3.5×10^5	$2.0 \times 10^5 \pm 2 \times 10^4$

S.E*=standard error of mean.

Table (4): incidence of Salmonella spp., Staph aureus and Clostridium perfringens species in the examined samples of vacuum packaged meat products (n=30 of each).

Examined samples	Sausage		luncheon	
	NO.	%	NO.	%
Salmonella spp	3	10	1	3.3
<i>Staph. aureus</i>	2	6.6	3	10
<i>Clostridium perfringens</i>	10	33.3	13	43.3

Table (5): Serotyping of salmonella spp isolated from examined samples of vacuum packaged meat products (n=30 of each).

Serotype	Sausage	Luncheon	Antigenic structure	
			O	H
<i>S.typhimurim</i>	1	1	1;1,4[5],12	i :1,2
<i>S.Entritidis</i>	2	2	1.9.12	9,m:1,7

Table (6): incidence of lecithinase positive strains of clostridium perfringens in the examined samples of vacuum packaged meat products (n=30 of each).

sample	NO. examined samples	NO. positive samples	Lecithinase positive		Lecithinase Negative	
			NO.	%	NO.	%
Sausage	30	10	7	70	3	30
Luncheon	30	13	10	77	3	23

Table (7): typing of lecithinase positive of Clostridium perfringens isolated from the examined samples of vacuum packaged meat products (n=30 of each).

sample	NO. lecithinase positive	Type							
		A		B		C		D	
		NO.	%	NO.	%	NO.	%	NO.	%
Sausage	7	7	70	-	-	-	-	-	-
Luncheon	10	10	100	-	-	-	-	-	-

D not detected. Tables (4) reported that Staph aureus was isolated from 6.6% for sausage and 10% for luncheon.

4. DISCUSSION

Meat is considered as an important source of protein and a potential source of a disease if not properly prepared. If not good hygienic practices applied especially during preparation, handling and serving of meat for large groups of people, it will provide opportunities for the spread of food borne disease. So, the safety and hygienic quality of food determined by the presence of microorganisms. The total aerobic plate count gives an idea about the hygienic

measures applied through receiving, handling and preparation of meat meals. So, it is the most reliable method for detection of sanitary levels of proper treatment of these food stuffs. It is evident from the results recorded in table (1) that the APC of the examined samples of vacuum packaged meat products ranged from 1.1×10^3 to 4.5×10^7 with an average of $2.1 \times 10^6 \pm 1.5 \times 10^6$ for sausage and 1.1×10^5 to 6.2×10^5 with an average of $2.9 \times 10^5 \pm 2.6 \times 10^4$ for luncheon respectively.

According to the safe permissible limit stipulated by ESO (2005) No (1090-2005) for APC in meat which was (not exceed 10^5 and 10^6 cfu /g), it was indicated that most of the examined samples come in accordance with this limit in corresponding to meat samples. Similar results were recorded by Fliss *et al.* (1991) (3×10^5)

however lower result were recorded by Eltaher (2009) (8.17×10^4) and Arab (2010) (2.4×10^5), Higher results recorded by Mansour (1995) (5.72×10^5) and Hashem (2015) ($6.18 \pm 0.67 \log 10$).

Table (2) indicated that total anaerobic plate count of the examined samples of vacuum packaged meat products was 1.1×10^5 to 7.1×10^7 with an average of $1.5 \times 10^7 \pm 3.5 \times 10^6$ for sausage and 1.2×10^5 to 4.2×10^5 with an average of $2.4 \times 10^5 \pm 2.5 \times 10^4$ for luncheon respectively. Lower result was recorded by (Hassan, 2009) (8.7×10^3).

Food sometimes contains bacteria of uncertain significance to human health, some of these bacteria belong to the normal intestinal flora of man and animals and many of them appear as food contaminants which lead to food spoilage (Warries, 2000).

Table (3) reported the total Enterobacteriaceae count of the examined samples of vacuum packaged meat products varied from 3.6×10^2 to 7.8×10^4 with an average $1.7 \times 10^4 \pm 3.9 \times 10^3$ for sausage and 1.1×10^5 to 3.5×10^5 with an average $2 \times 10^5 \pm 2 \times 10^4$ for luncheon samples. Nearly similar results were recorded with Hussein (1996) (1.9×10^5), and Daif (1996) (1×10^5), Lower results found by Hassan. (2015) ($2.2 \times 10^4 \pm 9.4 \times 10^3$), Capita *et al.* (2002) (3.38×10^3) But Hashem. (2015) ($3.91 \pm 0.96 \log 10$). and Vural (2007) (6.03×10^5) recorded higher results.

High Enterobacteriaceae count was more recorded in meat mainly due to contamination. It begins from the point of slaughtering until receiving of meat. Improper cooking does not kill chicken and all microorganisms and keeping this food in improper temperature leading to growth and proliferation of pathogenic organisms including Enterobacteriaceae group members.

The presence of Enterobacteriaceae in meat indicates a microbial proliferation, which could allow a 'multiplication of pathogenic and toxigenic microorganisms constituting a public health hazard (ICMSF, 1978).

Figure (1) demonstrated the comparison between percentage of APC, anaerobic plate count and the Enterobacteriaceae count for all examined samples of vacuum packaged meat products of sausage and luncheon It is appeared that total APC in 100% of all examined samples of vacuum packaged meat products of sausage and luncheon and followed by total Enterobacteriaceae account which appear by 73.3% in sausage and 66.6% in luncheon then total anaerobic appear by 60% in sausage and 70% in luncheon.

From this comparison, it is clear appeared that contamination was occurred

Tables (4) revealed that the incidence and serotyping of *Salmonella* isolated from the examined samples by 10% for sausage and 3.3% for luncheon.

Table (5) was identified serologically as *S.typhimurium* (1%) and *S.entretidis* (2%) . Results nearly similar to resulted recorded by Mansour (1995), and Khallaf (2014). But higher results reported by (Arumugaswamy *et al.*, (1993) and Hashem. (2015). Lower results were recorded by Jordan, *et al* (2006). These results agree with Khallaf (2014), Hashem (2015) and others and disagree with Ouf (2001), Reham (2004) and Arab (2010) .

Salmonellosis is a great problem and important food born disease. Mishandling in preparation of food of animal origin was the major reason for the outbreak of Salmonellosis (Rachimanin and Koulikouskii, 1990). The number of human cases of Salmonellosis increased due to serious hygienic diffeciency in a food technology during processing, production and storage of food as well as due to poor hygiene of a personal working (Koutikoyski and Kasijanenko, 1991).

Table (5) referred to the *C.perfringens* isolated from the examined vacuum packaged meat samples with 33.3% for sausage and with 43.3% for luncheon. Nearly similar results were obtained by Hassan (1998) while, Higher results recorded by Gutierrez *et al* (1999) and lower than by Miwa *et al.* (1998). These results agree with Shlatout (1999) and Atwa and EL-Roos (2011) and disagree with Hashem (2015) who not detect *C.perfringens*.

Labbe (2000) reported that processed meat was important as the most common food vehicle of *C. perfringens* type A food poisoning.

Table (6) reported the incidence of Lecithinase +ve strains in the examined samples of sausage were 7 of 10 (70%) of *C. perfringens* and Lecithinase -ve strains were 3 of 10 (30%).

Lecithinase +ve strains of *C. perfringens* in the examined samples of luncheon were 10 of 13 (77%) and Lecithinase -ve strains were 3 of 13 (23%).

It is noticed that there was a contamination and recontamination with *C.perfringns* in meat meals either before and after cooking especially in the presence of a lot of worker hands that dealing with handling cutting and preparing of the meat meals in a place serving a large number of people in a certain time.

The results reported in table (7) showed that +ve lecithinase strains of *C.perfringens* isolates were typing into type A,B,C and D toxins. Type A appeared in 70% of +ve Lecithenase in sausage samples and 100% in luncheon samples and B, C, D not detected. The results agree with Labbe (2000) who had demonstrated that raw meat and processed one were important as the most common food vehicle of *C. perfringens* type A food poisoning.

Tables (4) reported that *Staph aureus* was isolated from 6.6% for sausage and 10% for luncheon, respectively. These results nearly similar to Arab (2010) while higher results were achieved by Hashem (2015) and El Taher (2009) and lower results were recorded by Lotfi (1990).

Concerning to the obtained results, it could be concluded that raw meat samples were the most contaminated with *S. aureus* than other samples. This may reflect a bad hygienic practice during different stages from slaughtering, handling practices transportation and excessive handling during preparation of meals. A presence of this microorganism in post processing meat meal indicated that post processing contamination occur.

Contamination with *S. aureus* is an important risk index in evaluation of safety and hygienic quality of chicken meat (Jyhshiu *et al.*,2009). The presence of *S aureus* in heat treated food may be due to its contamination from food handlers, inadequate cleaned equipment or post processing contamination (Duffy *et al.*,2000).

Staphylococcus. aureus present on the nose and skin can be transferred to foods by handling. Subsequent storage enables growth and toxin production by the organisms (Roberts, 1990).

The obtained results in the current study concluded that the examined vacuum packaged meat product samples (sausage- luncheon) exposed for contamination during processing from point of slaughtering, handling, transportation, storage until to receiving which increased its contamination. Cooking especially boiling plays a great role in killing of most of these microorganisms

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