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MICROBIOLOGICAL ASPECTS OF SHEEP AND CATTLE MEATS IN EL-BEHERIA PROVINCE

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ABSTRACT

A total of 200 random samples of mutton and beef (100 of each meat type which were subdivided into 50 from each fore and hind quarters) were collected from butchers shops from El-Beheria province to show their microbiological aspects. The means of counts (cfu/g) of total aerobic bacterial, total Psychrotrophic bacteria, *Enterobacteriaceae, Coliforms, Staphylococcus aureus, Mould and Yeast* in mutton samples (fore and hind quarters) were $6.5\times10^6\pm1.4\times10^6$ & $5.8\times10^6\pm1.4\times10^6$, $3.2\times10^6\pm5.5\times10^5$&$4.6\times10^6\pm1.1\times10^6$, $6.2\times10^5\pm1.3\times10^5$& $6.1\times10^5\pm4.0\times10^4$, $5.0\times10^5\pm1.3\times10^5$&$5.9\times10^5\pm1.3\times10^5$, $2.2\times10^6\pm1.0\times10^6$& $3.89\times10^6\pm1.1\times10^6$, $6.1\times10^4\pm1.5\times10^4$ & $1.6\times10^5\pm6.0\times10^4$, $4.1\times10^4\pm9.1\times10^3$ & $1.2\times10^4\pm1.9\times10^3$ cfu/g respectively. While the means of these microbial counts (cfu/g) in examined beef samples (fore and hind quarters) were $1.4\times10^6\pm3.3\times10^5$ & $9.6\times10^5\pm1.6\times10^5$, $6.5\times10^5\pm1.6\times10^5$ & $1.2\times10^6\pm2.2\times10^5$, $8.5\times10^4\pm3.8\times10^4$ & $2.1\times10^6\pm9.3\times10^5$, $5.8\times10^5\pm1.4\times10^5$ & $3.9\times10^4\pm1.3\times10^4$, $5.9\times10^4\pm3.5\times10^4$ & $1.6\times10^5\pm7.6\times10^4$, $2.2\times10^4\pm5.8\times10^3$ & $1.8\times10^4\pm3.0\times10^3$, $8.6\times10^3\pm1.4\times10^3$ & 2.8×10^5 $\pm1.9\times10^4$ cfu/g respectively. Coliforms microorganisms were isolated by different percentage from examined mutton (fore and hind quarters) as follow: *E.coli* (Fecal&Non fecal) 44% and 49%, *Citrobacter spp*. 24% and 26%, *Klebsiella spp*. 23% and 14% and *Entrobacter aerogenes* 9% and 11%. While the same microorganisms were isolated from examined beef samples (fore & hind quarters) as 43% and 51%, 28% and 24%, 21% and 19%, 9% and 6%, respectively. *Salmonellae* could not be detected in both mutton and beef. This study was shown that the examined mutton samples were more contaminated than those of beef. The results were discussed from the hygienic view and compared with the national and international acceptable standards to assess their reliability for consumption.

Key words: *Cattle meats, Sheep, Mutton.*

INTRODUCTION

Raw meat is an ideal medium for bacterial growth; this is due to its high moisture contents. It is rich in protein, fermentable carbohydrate (glycogen), favourable pH and other growth factors (Magnus, 1981). Mayr *et al.* (2003) showed that meat provides an ideal condition for the growth of different spoilage bacteria thus making meat very perishable. Meat has long been known for its nutritive composition which could explain why it is being consumed by many people worldwide. The protein profile of meat consists of amino acids that have been described as excellent due to the presence of all essential ones required by the body. A large proportion of the world's populations rely on meat as a source of food. Enteric bacteria species can cause infections in humans when undercooked meat products are consumed (Collins and Thato, 2011). It has also been proved that protein and vitamins (especially A and B12) in meat could not be substituted for by plant sources, further justifying the nutritive importance of the former.

In Egypt, small ruminants (sheep) are slaughtered mostly during Islamic festivals and Christmas. Mutton and chevon are therefore popular meats in Egypt.

Consumer awareness for food that is microbiologically safe is increasing tremendously in developed countries, which is not the case observed in most developing countries. Therefore there is the need to produce meats that are of better quality and disease free especially in most developing countries. Food safety depends on their adequate manipulation, transportation and storage. Children, elderly and immunosuppressed individuals are particularly susceptible to foodborne infections than others.

Animals are slaughtered in Egypt abattoirs which are under standard and operated without adequate quality control systems and sometimes in backyards without observing strict hygienic practices. It is also a common practice to see people carrying carcasses just after dressing on their bare shoulders. Meats are normally transported to the butcher's shops either in meat vans, taxi's, motor cycle and bicycles. Meats are sold in the open butcher's shops sometimes in sieves or without sieves, and deboned on tables that are not well maintained or cleaned after work. Butchers and meat sellers pay little attention to their personal hygiene and serve meats with dirty hands and clothing's.

Meat is not only highly susceptible to spoilage, but also frequently implicated to the spread of food-borne illness, various biochemical changes and microorganisms are associated with meat, during the process of slaughter, processing and preservation (Olaoye and Nilude, 2010). Approximately 69% of gram negative bacteria are known to cause bacterial food borne disease (Okonko *et al.,* 2008a). Several researchers have reported that the meats samples were contaminated with high level of *Klebsiella pneumoniae, Enterobacter sp, Pseudomonas aeruginosa, E. coli, Salmonella sp, Serratia marcescens and Proteus vulgaris, Staphylococcus aureus and Bacillus sp* (Okonko *et al.,* 2010), (Collins and Thato, 2011). On the other hand, food-borne pathogens are able to disseminate from contaminated meat to the surfaces (Gorman *et al.,* 2002) and can spread infections in the community.

Meat foods are sometimes contaminated with germs after leaving the manufacture plant. Usually, hygiene conditions are poor when foods are produced in non-industrial establishments, mainly due to the fact that the necessary infrastructure for technologically adequate processes is not available. The wide range of contamination sources leads to the presence of a variety of microorganisms in food, among others, bacteria belonging to the genera *Escherichia, Salmonella* and *Staphylococcus,* in addition to various molds.

High contamination level of Coliforms in examined meat products may indicates unsanitary conditions of raw meat production from which produced. They are indicators of fecal pollution at slaughterhouse which begin from skinning and direct contact with knives and workers hands. Also, during evisceration and washing, contamination may come from intestinal contents as well as from water during rinsing and washing of carcasses. Undercooked meat products have caused many food poisoning incidents associated with *Escherichia coli* which is present in the faeces, intestines and hide of healthy cattle from where it can potentially contaminate meat during the slaughtering process (Duffy *et al.,* 2003). Coliforms count is a reliable indicator of inadequate processing and post processing contamination of such products (ICMSF, 1996). In addition, Coliforms in meat may be responsible for inferior quality resulting in economic losses beside their presence in high count may give rise to public health hazard (Moreno *et al.,* 1997).

International food management agencies, especially the World Health Organization (WHO), the Food and Agriculture Organization and the International Hazard Analysis Critical Control Point (HACCP) Alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices (GMPs).

This study was therefore undertaken to study the microbiological aspects and to assess the levels of microbial contamination in mutton and beef retailed in butchers shops of El-Beheria Province, Egypt.

MATERIALS and METHODS

1. Collection of samples:

A total of 200 random samples (250g weight of each) of retailed fresh meats represented by beef and mutton (100 of each subdivided into 50 from each fore and hind quarters) were collected aseptically from different butcher's shops at Al-Beheria province. The samples were placed separately in clean sterile plastic bags and transferred in an insulted ice box to the laboratory without delay under complete aseptic conditions. All collected samples were subjected to microbiological examination.

2. Methods:

2.1. Preparation of samples for microbiological examinations (ICMSF, 1978):

Tenth fold serial dilutions were used for counting of microorganisms under complete aseptic conditions, Ten grams of each collected sample were transferred into a sterile homogenizer flask containing 90ml of 0.1% sterile peptone water, the contents were homogenized for 2- 4 minutes at 1400 r.p.m and then allowed to stand for about 5minutes at room temperature to make the first serial dilution, 10^{-1} , the contents of the flask were thoroughly mixed by shaking and 1 ml was transferred into a separate sterile test tube containing 9 ml of 0.1% sterile peptone water to make the 2nd serial dilution, 10^{-2} , and so on to the dilution of 10^{-10}.

2.2. Microbiological examinations:

2.2.1. Determination of total aerobic bacterial count (TAC):

The total aerobic bacterial count was carried out by using standard plate count agar medium (Cruickshank *et al.,* 1975).

One ml from each of the previously prepared serial dilutions was aseptically transferred to duplicated plates of sterile Petri dishes, and then about 15 mls of sterile standard plate count agar previously melted and cooled at 45°C were poured and thoroughly mixed in a horizontal position. After solidification inoculated plates as well as control one were incubated in an inverted position at 37°C for 24-48hrs. Then the counted colonies were calculated as cfu/g and recorded.

2.2.2. Determination of total Psychrotrophic bacterial count:

The same steps as in TAC were carried out, than the plates were incubated at 7°C for 10 days. The average total Psychrotrophic count per gram was then calculated and recorded.

2.2.3. Determination of total Enterobacteriaceae count (ICMSF, 1978):

One ml from each of the previously prepared serial dilutions was aseptically transferred into two separate sterile plates of approximately 15 mls of sterile melted and tampered violet red bile glucose agar media (VRBG) were added. After solidification, thin layer of VRBG agar was overlaid (Gork, 1976). The plates were incubated at 37°C for 24-48 hrs. All purple colonies which surrounded by a purple zone were counted and the average number of colonies was determined and the Enterobacteriaceae count was calculated as cfu\g.

2.2.4. Isolation and identification of Coliforms (ICMSF, 1978):

2.2.4.1. Total Coliforms count:

One ml from each of the previously prepared serial dilutions were inoculated into duplicate plates of sterile melted and tempered violet red bile agar media (VRB) (45°C). After through mixing, each plate was allowed to solidify before being incubated at 37°C for 24hrs. All dark red colonies measuring 0.5 m.m or more in diameter were then counted and the average numbers of colonies were determined and so the Coliform count per gram was calculated. Suspected colonies were stabbed in semi-solid agar for further identification.

2.2.4. 2. Identification of suspected Coliform colonies (Ljutov, 1961, Simmons, 1926, Kovac`s 1928 and Christensen, 1946):

2.2.5. Isolation and identification of Staphylococcus aureus:

2.2.5.1. Staphylococcus aureus count (ICMSF, 1978):

From each of the previously prepared serial dilutions 0.1 ml was inoculated onto the surface of duplicate Baired Parker agar plates and was spreaded with a sterile bented glass rod until the surface of the medium was dried. The plates were incubated in an inverted position at 37°C for 48 hrs. All black shiny colonies with narrow white margins and surrounded by clear zones extended into the opaque medium were counted. Suspected colonies were stabbed in semi-solid agar for further identification.

2.2.5.2. Identification of staphylococcus aureus ((ICMSF, 1978, Cruickshank *et al.*, 1975):

2.2.6. Detection of Salmonellae (AOAC, 1984):

25 grams of each collected sample were transferred into a sterile homogenizer flask containing 225ml of 1.0% sterile peptone water and incubated for 24h/37°C then take 1ml was aseptically inoculated into 10ml of Rappaport Vassiliadis broth tubes and thoroughly mixed before being incubated at 43°C for 18 hours. A loopful from the Enriched Rappaport Vassiliadis broth culture was streaked onto duplicate plates of Salmonella Shigella agar (S.S). The inoculated plates were incubated at 37°C for 24 hours. Suspected colonies of non-lactose fermenters according to their culture behaviors were stabbed in semi-solid agar tubes for further identification.

3.2.2.6.1. Identification of suspected Salmonellae (Cruickshank *et al.*, 1975; Edward and Ewing, 1972).

2.2.7. Determination of total Mould and Yeast count (Baily and Scott, 1978):

The total Mould and Yeast counts was done by using Sabourad`s dextrose agar medium (Cruickshank, 1975), supplemented with chloramphenicol and chlortetracycline (100mg of each) as described by Koburger (1970).

Statistical analysis was made using sat 2004 T-test Annova test.

RESULTS

Table 1: Statistical analytical results of microbiological counts (cfu/g) of examined fore and hind quarter's mutton samples (50 of each).

	Fore quarter			Hind quarter		
	No.of +ve samples		X ±SEM	No. of +ve samples		X ±SEM
	No.	%		No.	%	
T. aerobic bacteria	50	100	$6.5 \times 10^6 \pm 1.4 \times 10^6$	50	100	$5.8 \times 10^6 \pm 1.4 \times 10^6$
T. Psychrotrophic bacteria	50	100	$3.2 \times 10^6 \pm 5.5 \times 10^5$	50	100	$4.6 \times 10^6 \pm 1.1 \times 10^6$
T. Enterobacteriaceae	47	94	$6.2 \times 10^5 \pm 1.3 \times 10^5$**	47	94	$1.1 \times 10^5 \pm 4.0 \times 10^4$**
Coliforms	45	90	$5.0 \times 10^5 \pm 1.3 \times 10^5$	47	94	$5.9 \times 10^5 \pm 1.3 \times 10^5$
Staphylococcus aureus	46	92	$2.2 \times 10^6 \pm 1.0 \times 10^6$	46	92	$3.9 \times 10^6 \pm 1.1 \times 10^6$
Total mould count	48	96	$6.1 \times 10^4 \pm 1.5 \times 10^4$	47	94	$1.6 \times 10^5 \pm 6.0 \times 10^4$
Total yeast count	47	94	$4.1 \times 10^4 \pm 9.1 \times 10^3$**	47	94	$1.2 \times 10^4 \pm 1.9 \times 10^3$**

Egyptian standard (E.S) of fresh meat no 4334/2004 stated that total aerobic bacterial count must not exceed 10^6.
T. =total No. =number X= mean SEM =standard error of mean
(**): Means with the same symbols were high significantly different ($p < 0.01$).

Table 2: Statistical analytical results of microbiological counts (cfu/g) of examined fore and hind quarters beef samples (50 of each).

	Fore quarter			Hind Quarter		
	No. of +ve samples		X ±SEM	No. of +ve samples		X ±SEM
	No.	%		No.	%	
T. aerobic bacteria	50	100	$1.4 \times 10^6 \pm 3.3 \times 10^5$	50	100	$9.6 \times 10^5 \pm 1.6 \times 10^5$
T. Psychrotrophic bacteria	50	100	$6.5 \times 10^5 \pm 1.6 \times 10^5$	50	100	$1.2 \times 10^6 \pm 2.2 \times 10^5$
T. Enterobacteriaceae	44	88	$8.5 \times 10^4 \pm 3.8 \times 10^4$*	49	98	$2.1 \times 10^6 \pm 9.3 \times 10^5$*
Coliforms	44	88	$5.8 \times 10^5 \pm 1.4 \times 10^5$***	46	92	$3.9 \times 10^4 \pm 1.3 \times 10^4$***
Staphylococcus aureus	43	86	$5.9 \times 10^4 \pm 3.5 \times 10^4$	48	96	$1.6 \times 10^5 \pm 7.6 \times 10^4$
Total Mould count	43	86	$8.6 \times 10^3 \pm 1.4 \times 10^3$ **	45	90	$2.8 \times 10^5 \pm 1.9 \times 10^4$ **
Total Yeast count	45	90	$2.2 \times 10^4 \pm 5.8 \times 10^3$	47	94	$1.8 \times 10^4 \pm 3.0 \times 10^3$

T. =total No. =number X= mean SEM =standard error of mean
Mean bearing different symbols are significantly different at (***) differ significantly at ($p<0.0001$).
(**) differ significantly at ($p<0.001$)
(*) differ significantly at ($p<0.05$).

Table 3: Frequency distributions of microbial counts of examined fore and hind quarters mutton samples (n=50 for each of fore and hind).

		0-10		>10-10^2		>10^2-10^3		>10^3-10^4		>10^4-10^5		>10^5-10^6		>10^6-10^7		>10^7-10^8	
		No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
TAC	Fore	0	0	6	12	10	20	13	26	14	28	5	10	2	4	0	0
	Hind	0	0	6	12	10	20	10	20	15	30	5	10	2	4	0	0
Enterobacteriaceae	Fore	3	6	11	22	12	24	10	20	8	16	6	12	0	0	0	0
	Hind	3	6	15	30	12	24	12	24	5	10	3	6	0	0	0	0
Coliforms	Fore	5	10	14	28	10	20	7	14	6	12	8	16	0	0	0	0
	Hind	3	6	15	30	12	24	8	16	5	10	7	14	0	0	0	0
Staph.aureus	Fore	4	8	16	32	12	24	8	16	4	8	6	12	0	0	0	0
	Hind	4	8	13	26	12	24	8	16	5	10	5	10	3	6	0	0

Table 4: Frequency distributions of microbial counts of examined fore and hind quarters beef samples (n=50 for each of fore and hind).

		0-10		>10-10^2		>10^2-10^3		>10^3-10^4		>10^4-10^5		>10^5-10^6		>10^6-10^7		>10^7-10^8	
		No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
TAC	Fore	0	0	8	16	12	24	16	32	6	12	6	12	2	4	0	0
	Hind	0	0	2	4	15	30	22	44	5	10	6	12	0	0	0	0
Enterobacteriaceae	Fore	6	12	14	28	12	24	8	16	6	12	4	8	0	0	0	0
	Hind	1	2	18	36	10	20	12	24	9	18	0	0	0	0	0	0
Coliforms	Fore	6	12	12	24	14	28	6	12	7	14	5	10	0	0	0	0
	Hind	4	8	12	24	14	28	10	20	4	8	6	12	0	0	0	0
Staph.aureus	Fore	7	14	13	26	10	20	10	20	4	8	6	12	0	0	0	0
	Hind	2	4	15	30	10	20	12	24	4	8	7	14	0	0	0	0

Table 5: Incidences of isolated Coliform microorganisms in examined mutton and beef samples.

Bacterial species		Mutton				Beef			
		Fore quarter		Hind quarter		Fore quarter		Hind quarter	
		No.	%	No.	%	No.	%	No.	%
E.coli	*Non feacal*	10	22	10	21	8	18	11	23
	True feacal	10	22	13	28	11	25	13	28
Citrobacter diversus		6	13	5	11	5	12	5	11
Citrobacter frundii		5	11	7	15	7	16	6	13
Klebsiella oxytoca		4	9	3	6	4	9	5	11
Klebsiella pneumoniae sub.pneumoniae		6	14	4	8	5	12	4	8
Enterobacter aerogense		4	9	5	11	4	9	3	6

Table 6: Comparsion between means of microbial loads in examined beef and mutton sample

	Beef meat	**Mutton meat**
Total aerobic bacterial count(TAC)	$1.1\times10^6\pm1.8\times10^5$***	$6.2\times10^6 \pm 9.6\times10^5$***
Total Psychrotrophic count(TPC)	$9.1\times10^5\pm1.3\times10^5$***	$3.9\times10^6\pm6.3\times10^5$***
Enterobacteriaceae count	$1.1\times10^6\pm4.8\times10^5$	$3.6\times10^5\pm7.6\times10^4$
Coliform count	$3.0\times10^5\pm7.6\times10^4$*	$5.5\times10^5\pm9.4\times10^4$*
Staphylococcus aureus count	$1.1\times10^5\pm4.3\times10^5$**	$3.0\times10^6\pm7.4\times10^5$**
Mould count	$1.5\times10^5\pm1.7\times10^4$	$1.1\times10^5\pm3.0\times10^4$
Yeast count	$2.0\times10^4\pm3.2\times10^3$	$2.6\times10^4 \pm 4.8\times10^3$

Means bearing different symbols are significantly different at:
(***) difference highly significant ($p<0.0001$). (**) difference moderatly significant ($p<0.001$).
(*) difference slightly significant ($p<0.05$).

DISCUSSION

Conditions of the animals prior slaughtering have an impact on the microbial load of meats. Sources of microbial contaminations of carcasses include: the animal (hides and gastro-intestinal tract), workers, utensils, equipment, air and water. Hence the level of microbial contaminations of a carcass at this stage depends upon the degree of sanitation practiced during the slaughtering- dressing procedures. Because of location and handling practices certain areas of a carcass are more likely to be contaminated or to remain contaminated than are others. For these reasons, microorganisms are not uniformly distributed over the carcass (NAS, 1985).

It is evident from Table (1) that the means of total aerobic mesophilic and Psychrotrophic bacterial counts (cfu/g) of examined samples of fore quarters meat of sheep carcasses were $6.5\times10^6\pm1.4\times10^6$ and $3.2\times10^6\pm5.5\times10^5$, while these of hind quarters meat samples were $5.8\times10^6 \pm 1.4 \times10^6$ and $4.6\times10^6\pm 1.1\times10^6$, respectively.

Higher total bacterial counts were reported by Al-Aboudi and Hamed (1988) who revealed that the mean aerobic bacterial count of sheep carcasses slaughtered at Mosul-abattoir-Iraq was 4.7×10^7, where 35% of the examined carcasses had counts more than 10^7.while 54% had counts ranged from 10^6to10^7/g.

Lower total bacterial count was reported by Bhagirthi *et al.* (1983) who reported that the market fresh mutton samples had bacterial counts between 10^4 to 10^5/g. Only 4% of each of fore and hind quarters meat samples were exceeded the acceptable limit (10^6) for total aerobic bacterial counts established by the Egyptian standard (E.S.) No. 4334/2004 (table, 3) and that set by the International Commission on Microbiological Specification (ICMS, 1982) (<1.0×10^6 cfu/g) (Table, 3).

The high counts of total aerobic bacteria may be due to the manual dressing of carcass hides with the hands of the abattoir workers (Elliott and Michener, 1961). Usually, hygiene conditions are poor when foods are produced in non-industrial establishments, mainly due to the fact that the necessary infrastructure for technologically adequate processes is not available. Spoilage or reduce keeping life of

fresh meat can be generally attributed to the presence of very large number of bacteria, these were mainly identified as members of Psychrotrophic bacteria and certain other microorganisms capable of growing at 0°C (Mousa *et al.,* 1988).

Also, Table (1) showed that the means of total *Enterobacteriaceae* counts (cfu\g) of examined samples of fore and hind quarters meats of sheep carcass were $6.2\times10^5\pm1.3\times10^5$ and $1.1\times10^5\pm4.0\times10^4$ but the means for their Coliforms counts were $5.0\times10^5 \pm 1.3\times10^5$ and $5.9\times10^5 \pm 1.3\times10^5$, respectively. Their incidences were equal (94%) in hind quarters but were different in fore ones (94% and 90%, respectively).

Most of the enterobacteria present in meats come from faecal contaminations. Elevated numbers of enterobacteria can be an indicator of poor hygienic conditions during handling or inadequate storing (Vanderlinde *et al.,* 1998).

It is evident from Table (1) that the means of *Staph. aureus* counts of fore and hind quarters of examined samples of sheep carcass were $2.2 \times 10^6 \pm 1.0 \times 10^6$ and $3.9 \times 10^6 \pm 1.1 \times 10^6$, with equal incidences of each (92%), respectively.

Only 40% & 34% of the examined fore and hind quarters meat samples were not exceeded the acceptable limit ($<1.0 \times10^2$ cfu/g) recommended by ICMS (1980) for *Staph. aureus* count.

High contamination level of Coliforms in examined meat products may indicates unsanitary conditions of raw meat production from which produced. They are indicators of fecal pollution at slaughterhouse which begin from skinning and direct contact with knives and workers hands. Also, during evisceration and washing, contamination may come from intestinal contents as well as from water during rinsing and washing of carcasses. Undercooked meat products have caused many food poisoning incidents associated with *Escherichia coli* which is present in the faeces, intestines and hide of healthy cattle from where it can potentially contaminate meat during the slaughtering process (Duffy *et al.,* 2003). Coliforms count is a reliable indicator of inadequate processing and post processing contamination of such products (ICMSF, 1996). In addition, Coliforms in processed meat may be responsible for inferior quality resulting in economic losses beside their presence in high count may give rise to public health hazard (Moreno *et al.,* 1997).

Studies indicated that large numbers (usually $>10^6$ cfu/g) of coagulase positive *Staph.aureus* must contaminate the food for producing sufficient enterotoxin to cause food poisoning (Liston *et al.,* 1971; Gilbert *et al.,* 1972).

In this study, Salmonellae could not be isolated either from fore and hind quarters mutton samples.

Table (1) showed that the means of Mould and Yeast counts (cfu/g) in fore quarters meat samples were $6.1\times10^4 \pm1.5\times10^4$ and $4.1\times10^4 \pm 9.1\times10^3$ whereas in hind quarters,they were $1.6\times10^5 \pm 6.0\times10^4$ and $1.2\times10^4\pm1.9\times10^3$, respectively. The short life of meat (about 4 days for fresh chilled meats according to E.S. No. 4334/2004) limits the possibility of fungal contamination; however, molds and yeasts are widely distributed in the environment and can easily reach the meats through contaminated equipments or air, thus leading to alterations of the meat product that can provoke infections or allergic reactions.

Salmonella, Staphylococcus spp. and *E.coli* infections infections can be contracted through consumption of contaminated mutton. *Salmonellae* are important causes of gastroenteritis. Symptoms of *Salmonella* infection in healthy human-beings include fever, diarrhea, abdominal pain, and sometimes vomiting. *Staphylococcus* spp. can be part of normal flora on the skin of humans and animals which can be transmitted from person to product through unhygienic practices (Postgate, 2000). *Staphylococcus* spp. cause infections such as arthritis, black pox, boil, bronchitis, bumble foot, carbuncle, cystitis, endocarditis, meningitis, osteomyelitis, pneumonia, and scalded skin (Stuart, 2005). Others cause food poisoning resulting in severe vomiting and diarrhea. *Escherichia coli* causes illness ranging from gastrointestinal tract-related complications such as diarrhea, dysentery, urinary tract infection, pneumonia and even meningitis (Johnson *et al.,* 2006), although majority of the *Escherichia coli* strains are non-pathogenic and exists in the intestinal tract of humans and animals.

Concerning the beef sample, the data tabulated in Table (2) was showed that the means of total aerobic bacterial and Psychrotrophic counts (cfu/g) of fore quarters meat samples for cattle carcasses were $1.4\text{x } 10^6\pm 3.3 \text{ x } 10^5$ and $^{6.5}\text{ x}10^5\pm 1.6 \text{ x } 10^5$ and these of hind quarters were $9.6 \text{ x } 10^5\pm 1.6 \text{ x } 10^5$ and $1.2\text{x } 10^6 \pm 2.2 \text{ x } 10^5$, respectively.

Nearly similar results were recorded by El-Said (1992) and Mansou (1995), but higher results were recorded by Elwi (1994) and Abdel Aziz (1997). Lower results were registerated by Marouf (1989).

As in mutton, only 4% of fore quarters meat samples taken from cattle carcasses were exceeded the acceptable limit (10^6) for total aerobic bacterial counts established by the Egyptian

Standard Specification "ESS"(No, 4333/2004) and that set by the International Commissions on Microbiological Specifications (ICMS, 1980) (<1.0 $\times 10^6$ cfu/g) but these taken from hind quarters were agree with them (Table, 4).

Niamy *et al.* (1997) suggested that meat safety could be improved by better hygienic conditions during slaughter and transport of the meat.

Spoilage of whole cuts of meats at refrigeration temperatures is primarily a surface phenomenon resulting in formation of slime and off-odour. The shelf life of raw chilled meat is prolonged by those factors affecting the growth rate of the pychrotrophs; dry surface, low initial level of pychrotrophs, the inherent pH of the meat, oxygen limition and temperature. Wraping meat in oxygen-impermeable films retards surface growth and selects for microaerophilic bacteria like *Lactobacillus* or *Brochothrix thermosphacta* at the expense of the *Pseudomonas-Acintobacter-Moraxella* group (Gardner, 1981).

Table (2) showed that the means of total *Enterobacteriaceae* counts(cfu\g) of examined samples of fore and hind quarters meats of cattle carcass were $8.5\times10^4 \pm 3.8\times10^4$ and $2.1\times10^6 \pm 9.3\times10^5$ but the means for their Coliforms counts were $5.8\times10^5 \pm 1.4\times10^5$ and $3.9\times10^4 \pm 1.3\times10^4$, respectively. Their incidences were equal (88%) in fore quarters but were different in hind ones (98% and 92%, respectively).

Similar results were obtained by El-Said (1992) who examined raw meats used in the production of fresh sausages. The mean of *Enterobacteriaceae* counts was $5 \times10^4 \pm 5 \times10^3$.

Table (2) showed that means of total *Staph.aureus* counts (cfu/g)of beef samples that were taken from fore and hind quarters were $5.9x10^4 \pm 3.5 \times 10^4$ and $1.6x\ 10^5 \pm 7.6 \times 10^4$; with incidences of 86% and 96%, respectively.

Lower results were registered by El-Taher (2009) {9.7×10^3 cfu/g with an incidence of 36.6% from raw meat} and Elwi (1994) {500 cfu/g}.
Although the raw fresh meats must be free from pathogenic microorganisms according to the *E.S. no. 4334/2004*, only 40% & 34% of examined beef samples of fore and hind quarters were complied with the acceptable limit (<1.0×10^2 cfu/g) recommended by ICMS (1980) for *Staph. aureus* count. Also, Table (2) was showed that the means of Mould and Yeast counts(cfu/g)in examined samples of fore quarters beef were $8.6 \times10^3 \pm 1.4\times10^3$ and $2.2 \times10^4 \pm 5.8\times10^3$ and of hind quarters were $2.8\times10^5 \pm 1.9\times 10^4$ and $1.8\times10^4 \pm 3.0\times 10^3$.

Yeasts and Moulds can cause various degrees of food decomposition. Appearance of contaminated food may range from no blemish to sever blemish to complete decomposition. Growth of yeasts and molds may be manifested as rot spots, pustules or scabs, slime, white or variously colored mycelia and spores. Some foodborne yeasts and molds are undesirable because of potential hazards to human and animal health (Beneke and Rogers, 1971). Numerous molds can produce mycotoxins (Mislivec, 1981).

Table (5) showed that the members of Coliforms group (E.coli, *Citrobacter, Klebsiella and Enerobacter*) were isolated from mutton with different percentages (from fore as 44%, 24%, 23% and 9% and from hind as 49%, 26%, 14% and 11%, respectively). *Escherichia coli* was occupied the first one with high percentages. It is often used as hygiene indicators of foods of animal origin. This is a highly recognized food pathogen that causes gastro-intestinal diseases in humans; its presence on processed food may give a better indication than Coliforms of inadequate treatment or post-process contamination from the environment, and may help to indicate the extent of faecal contamination (Nel *et al.,* 2004; Crowley *et al.,* 2005). Nel *et al.* (2004) has stated that the maximum limit of *E.coli* in meat and meat products should not be more than10 cfu/g (Mathenjwa, 2010). Also Table (5) showed that the incidences of *E.coli (*fecal and non-fecal origin*), Citrobacter spp., Klebsiella spp. and Enterobacter aerogense* in beef samples that were taken from fore quaters were as follow: 43%, 28%, 21% and 9%; but for that were taken from hind quarters were 51%, 24%, 19% and 6%, respectively. El-Taher (2009) isolated *E.coli* from 20% of the examined raw meats.

Salmonella spp. could not be also isolated from any of examined beef samples and this result was contrasted with that was detected by Fahem (1993).

However, *S. aureus* and *E. coli* could not pass the test of a 10^2cfu/g which the Egyptian Standards Board sets for fresh beef. The presence of *E. coli* in the meat samples is as a result of contamination with faecal matter which could be from the environment, air, materials used including water. The hands of the handlers or even the contents of portions of the meat like the intestines which appear to be the very immediate sources could also be implicated. From preliminary investigation conducted, the environments in which the meat was processed and sold were not hygienically maintained, thus the presence of the *E. coli*. The standard recommended by ICMS (1980) is <$1.0x10^2$ cfu/g normally, pathogens in general should have a 10^2cfu/g or no count in all ready to eat foods. Reference to the ICMS criteria may suggest that the pathogen levels in the beef are acceptable since they

would have been destroyed after processing at high temperatures. This notwithstanding there is a risk of infection if virulent forms of this bacterium are present and the beef which is not well processed before consumption.

Coliforms, E.coli, Staph.aureus and *Salomnella* are often present on fresh tissues because the slaughtering process does not include a bactericidal step. Levels of these bacteria on freshly slaughtered animal carcasses will be varied depending upon climatic, farm, livestock transport, stockyard and processing conditions. In general all of them except Salmonella may be present at levels of about 10 to 10^2 (Johnston and Tompkin, 1992).

Table (6) was showed the comparison between microbial loads of beef and mutton where the means values of total aerobic bacteria, total Psychrotrophic, *Coliforms* and *Staphylococcus aureus* counts were differ significantly as follow $1.1\times10^6\pm 1.8\times10^5$ vs vs $6.2\times10^6 \pm 9.6\times10^5$, $9.1\times10^5\pm 1.3\times10^5$vs $3.9\times10^6 \pm 6.3\times10^5$, $3.0\times10^5\pm7.6\times10^4$ vs$5.5\times10^5\pm 9.4\times10^4$, $1.1 \times10^5\pm4.3 \times 10^5$ vs $3.0\times10^6\pm7.4\times10^5$ cfu/g, respectively. This is due to contamination of the sheep carcasses during the slaughtering process. It was subjected to poor sanitary conditions prevailing at both abattoir and a butcher`s shops may be the main cause of high incidence of *E.coli* (El-Mosalami and Wassef, 1973). Also the sheep slaughtering steps contain more handling to the carcasses than those of cattle by intervention of the workers through hanging, pushing and trimming of the carcasses which increase Staphylococci. Also presence of the fleece which act as shedding source of microorganisms. Selvan *et al.* (2007) found that the mean of total aerobic bacterial counts was significantly greater in mutton products than all other products (beef) studied.

Generally, although high contamination levels with mesophilic aerobes have been reported for raw mutton and beef in this study, their counts were below the 10^6 cfu/g acceptable limit and they were below 10^7 where spoilage of meat occur (Warriss, 2001). The main factors might be the inadequate hygiene during slaughtering, processing and handling, moreover the heaps of garbage that were scattered from place to place beside our abattoirs. The isolation of *Staphylococcus aureus* and *E. coli* can be worrying because certain strains of these bacteria cause food-borne infections.

To reduce microbiological load on and in animals' carcasses, standard operating methods should be practiced. Such methods include screening of butchers, meat sellers and all who handle meat on regular basis on their health status. In addition well maintained meat vane, selling tables covered with nets, thoroughly cleaned and regularly sterilized knives, aprons and all equipments come into contact with meats should be used. Apart from these, meat cooked to an internal temperature of 70˚C for 15minutes will help in killing all bacteria before consumption.

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الجوانب الميكروبيولوجية للحوم الاغنام والابقار فى محافظة البحيرة

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أجريت هذة الدراسة على ٢٠٠ عينة من لحوم ذبائح الاغنام والابقار (١٠٠ عينة لكل نوع مقسمة الى ٥٠ لكل من الارباع الامامية والخلفية) المعروضة للبيع فى محلات الجزارة بمحافظة البحيرة وذلك لاستبيان النواحى الميكروبيولوجية بها. وقد تم اجراء الفحوص الميكروبيولوجية الاتية:العدد الكلى لكل من: الميكروبات الهوائية ؛ الميكروبات المحبة للبرودة ؛الميكروبات العصوية المعوية؛العصيات القولونية؛الميكروب العنقودى الذهبى؛ الفطريات والخمائر- بالاضافة الى الكشف عن تواجد ميكروب السالمونيلا. وكانت متوسطات اعداد هذة الميكروبات فى لحوم ذبائح الاغنام فى الارباع الامامية والخلفية كالاتى: $6.5\times10^6\pm 1.4\times10^6$ and $5.8\times10^6\pm 1.4\times10^6$, $3.2\times10^6\pm 5.5\times10^5$ and $4.6\times10^6\pm 1.1\times10^6$, $6.2\times10^5\pm 1.3\times10^5$ and $6.1\times10^5\pm 4.0\times10^4$, $5.0\times10^5\pm 1.3\times10^5$ and $5.9\times10^5\pm 1.3\times10^5$, $2.2\times10^6\pm 1.0\times10^6$ and $3.89\times10^6\pm 1.1\times10^6$, $6.1\times10^4\pm 1.5\times10^4$ cfu بينما كانت المتوسطات g respectively. /and $1.6\times10^5\pm 6.0\times10^4$, $4.1\times10^4\pm 9.1\times10^3$ and $1.2\times10^4\pm 1.9\times10^3$ فى لحوم ذبائح الأبقار (الأرباع الأمامية والخلفية) كالآتى: $1.4\times10^6\pm3.3\times10^5$ and $9.6\times10^5\pm1.6\times10^5$, $6.5\times10^5\pm1.6\times10^5$ and $1.2\times10^6\pm2.2\times10^5$, $8.5\times10^4\pm3.8\times10^4$ and $2.1\times10^6\pm9.3\times10^5$, $5.8\times10^5\pm1.4\times10^5$ and $3.9\times10^4\pm1.3\times10^4$, $5.9\times10^4\pm3.5\times10^4$ and $1.6\times10^5\pm7.6\times10^4$, $2.2\times10^4\pm5.8\times10^3$ and $1.8\times10^4\pm3.0\times10^3$, $8.6\times10^3\pm1.4\times10^3$ and $2.8\times10^5\pm1.9\times10^4$ cfu/ g respectively. هذا وقد تم عزل بعض الميكروبات القولونية بنسب مختلفة من لحوم الأغنام (الأرباع الأمامية والخلفية) وهى كالأتى:الاشريكية القولونية (البرازية والغير برازية) ٤٤ % و٤٩%، جنس ستروباكتر٢٤% و٢٦%، جنس الكلبسيلة ٢٣% و١٤% وانتيروباكتر ايروجينس ٩% و١١%. أما فى لحوم الأبقار فقد تم عزل مثيلاتها بالنسب الآتية: ٤٣% و٥١% ، ٢٨% و ٢٤% ، ٢١% و١٩% ، ٩ % و ٦ % على التوالى. لم يتم اكتشاف ميكروب السالمونيلا فى لحوم الاغنام والابقار. كما أوضحت هذة الدراسة ان لحوم الاغنام كانت اكثر تلوثا من لحوم الأبقار. وقد تم مناقشة النتائج من الناحية الصحية ومدى مطابقتها للمواصفات القياسية المصرية والعالمية ومدى ملاءمة هذة اللحوم للاستهلاك الادمى.