Occurrence of enterobacteriaceae in fresh camel meat with special reference to salmonellae

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A B S T R A C T

Eighty random samples of freshly slaughtered camel meat were collected from 4 different abattoirs namely (A) El-shohdaa, (B) El-Bagour, (C) Menouf and (D) Sadat (20 of each) located in Menofia governorate. and subjected to bacteriological examination .The obtained results declared that the mean Enterobacteriaceae counts in examined meat camel samples were recorded descendingly in different abattoirs as in B ($9.77 \times 10^7 \pm 3.44 \times 10^7$)then in D ($7.86 \times 10^7 \pm 4.91 \times 10^7$) then in A ($7.50 \times 10^7 \pm 4.08 \times 10^7$) then in C ($1.40 \times 10^7 \pm 6.54 \times 10^7$). Incidence and serotyping of *E.coli* isolated from the examined samples of camel meat from the different abattoirs were (O_{19}:H_{21}, O_{44}:H_{18} ,O_{86}, O_{111}:H_4,O_{114}:H_4,O_{124},O_{127}:H_6,O_{121}:H_7,O_{171}:H_2) with ratio (5 ,0 ,5 ,0 ,5 ,5 , 0 , 0, 0, 0) , (10 , 0, 0, 5 ,0 ,5 ,5 ,5 ,0) , (5 ,10 ,0 ,0 ,5 ,5 ,0 ,0 ,0) and (5, 10, 0 ,0 ,5 ,5 ,0 ,0 ,0) in A , B, C and D abattoirs, respectively . Salmonellae isolated from the examined samples of camel meat from the different abattoirs were S.Enteritidis, Typhimurium, Virchow, Heidberg ,Kentucky and Infantis with ratio (5% ,10% ,0% .0%,5% ,5%),(5% ,5% ,0% ,0% ,0% ,0%),(10% ,10% ,5% ,5% ,5% ,0%) and (5%,5%,0%,0%,5%,5%) in A , B,C and D abattoirs , respectively.

Keywords: *Camel meat, Enterobacteriacea, E.coli. Salmonella.*

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1. INTRODUCTION

Camel´s meat is considered as a good source of protein in some Arab countries that may play an appreciable role in solving food problems (Alao *et al.*, 2017).

No doubt that the microbiological contamination of carcasses occurs mainly during processing and handling, such as skinning, evisceration, preparation, storage and distribution at slaughterhouses and retail establishment (Edris *et al.*, 2013). Enterobacter species is among the most common Gram negative pathogens associated with hospital infections, representing 6 % of all nosocomial isolates recovered and 11 % of Pneumonia isolates (Musa *et al.*, 2017). *Klebsiella Pneumoniae* and *Klebsiella oxytoca* are also opportunistic pathogens that have been linked over the years as the main cause of diseases in humans such as septicemia, pneumonia, urinary tract and soft tissue infection moreover the Proteus Vulgaris an opportunistic pathogen in humans where it is also known to cause

urinary tract and wound infections (Struble *et al.,* (2009). Extra intestinal *E.coli* infections, including urinary tract infection, sepsis, and other extra intestinal infections occurred to human consuming contaminated food of animal origin but also Escherichia coli have public health impact on humans by causing gastroenteritis including hemorrhagic colitis and hemolytic uremic syndrome and isolation of *E. coli* with multidrug resistance from fast food and meat sources is a clear indication of environmental contamination and is a matter of public health concern. (Pavithra and Ghosh, 2013). Salmonellae are well-known pathogens, highly adaptive and potentially pathogenic for humans and/or animals. Salmonella infections are capable of producing serious infections that are often foodborne and present as gastroenteritis. However, a small percentage of these infections may become invasive and result in bacteremia and serious extra intestinal disease. In human beings, *S. typhimurium* usually causes an infection by the fecal-oral route and the symptoms are a self-limiting gastroenteritis with mild fever, diarrhea, abdominal pain, nausea and vomiting. In immune-compromised adults, it can cause a systemic infection with additional complications moreover, *S.typhimurium* can be shed in a patient's excretion for several weeks because it often remains in the intestine (Haimovich and Venkatesan, 2006). *Salmonella enterica* is a leading cause of human gastroenteritis in both developed and developing countries, causing millions of human and animal illnesses and significant economic losses worldwide. (EFSA, 2011) Multiplex PCR (mPCR) allows multiple gene analysis of bacteria at the same time in a single reaction tube simultaneously, saving time and reagents. A rapid, sensitive and specific method that would allow detection of multiple pathogens simultaneously from different types of foods would be very valuable for the food industry and regulatory agencies (Xu et *al.,* 2012).

2. Materials and methods

2.1. Sample collection:

A total number of 80 camel meat samples were collected immediately after slaughtering from 4 largest different abattoirs namely El-shohdaa (A) , El-Bagour (B) , Menouf (C) and EL-Sadat (D) which located in Menofia governorate. Each abattoir was represented by 20 samples of camel meat.

2.2. Preparation of samples (ICMSF, 1996):

Accurately, 25 gm of the examined camel meat samples were transferred to polyethylene bags, to which 225 ml of 0.1% of sterilized buffered peptone water (0.1%) were aseptically added to the content of the bag. Each sample was then homogenized for 2 minutes at 2500 r.p.m using a sterile homogenizer to provide a homogenate of 1/10 dilution. The mixture was allowed to stand for 15 minutes at room temperature then one ml from the original dilution was transferred by means of sterile pipette to another sterile tube containing 9 ml of sterile peptone water (1%) from which further serial decimal dilution were prepared.

The prepared samples were subjected to the following examinations:

2.3. Enterobacteriaceae count (ISO 2004):

The same technique of the previous pour plate method was applied using Violet Red Bile Glucose agar medium. The plates were incubated at 37 ºC for 24 hours. Suspected colonies, which showed purplish – red colonies surrounded by a red zone of precipitated bile acid, were enumerated to obtain total Enterobacteriaceae counts per gm.

2.4. Coliform count (ICMSF (1996) :

One ml of each previously prepared serial dilution was inoculated into a sterile labeled

Petri dish, using Pour plate method, add 15 ml of tempered melted Violet red bile agar (cooled to 44 – 46ºC) to each Petri dish, then thoroughly and uniformly mixed with the inoculum and left to solidify. When the agar is completely set add a further 10ml of tempered VRB onto the surface of the inoculated plate. After solidification, the inoculated plates were incubated at an inverted position at 37 ºC for 24 ± 2 hours. All dark red colonies measuring 0.5 mm or more in diameter on uncrowded plates were then counted and the average number of colonies was determined. The coliform count per gram was calculated.

2.5. Total Enterobacteriaceae count (ISO 2004):

The same technique of the previous pour plate method was applied using Violet Red Bile Glucose agar medium. The plates were incubated at 37 ºC for 24 hours. Suspected colonies, which showed purplish – red colonies surrounded by a red zone of precipitated bile acid, were enumerated to obtain total Enterobacteriaceae counts per gm.

2.6. Identification of family enterobacteriaceae:

Members belonging to Enterobacteriaceae were further identified according to Cowan and Steel (1974).

2.7. Screening for Enteropathogenic Escherichia coli:

Pre-enrichment:

From the original dilution, one ml was inoculated into MacConkey broth tubes supplemented with inverted Durham's tubes. Inoculated tubes were incubated at 37°C for 24 hours.

Enrichment broth:

One ml from positive MacConkey tube was inoculated into another MacConkey broth tubes and incubated at 44°C for 24 hours.

Plating media:

Loopfuls from positive MacConkey broth tubes were separately streaked onto Eosin Methylene Blue agar medium (E.M.B.), which was then incubated at 37°C for24 hours. Suspected colonies were metallic green in color. Suspected colonies were purified and inoculated into slope nutrient agar tubes for further Morphological and Biochemical identification.

Serodiagnosis of E.coli:

The isolates were serologically identified according to (Kok *et al.,* 1996) by using rapid diagnostic *E.coli* antisera sets (DIFCO Laboratories, Detroit Michigan 48232-7058, USA) for diagnosis of the Enteropathogenic types.

2.8. *Screening for Salmonellae:*

Pre-enrichment broth:

Twenty five grams of examined samples were homogenized in 225 ml of sterile peptone water and incubated at 37°C for 18 hours.

Enrichment broth:

One ml of the original dilution was inoculated into 9 ml Rappaport Vassilidis broth tube, and then the tube was incubated at 43°C for 24 hours (Harvey and Price, 1981).

Selective Plating:

Xylose lysine desoxychoclate agar (X.L.D) was used. Loopfuls from the inoculated tubes were separately streaked onto X.L.D. agar medium and incubated at 37°C for 24 hours. Suspected colonies were red with or without black centers .Suspected colonies were purified and inoculated into slope nutrient agar tubes for further Morphological and Biochemical identification.

Serological identification of Salmenollae:

Isolates proved biochemically to be Salmonella microorganisms were subjected to serological identification according to Kauffman white scheme (Kauffman, 1974)by using rapid diagnostic Salmonella antisera sets (Welcome Diagnostic, a Division of the Wellcome Foundation Limited, Dartford England DA15 AH).

2.9. Detection of Toxin producing genes in isolated salmonella strains using Multiplex PCR:

Primer sequences of Salmonellae used for PCR system:

The primers for detection of virulence factors including Enterotoxin (stn), hyper-invasive locus (hilA) and fimbrial (fimH) genes of Salmonellaspecies were synthesized as shown in the table 1.

DNA Extraction using QIA amp kit (Shah et al., 2009):

After overnight culture on nutrient agar plates, 1 or 2 colonies were suspended in twenty ml of sterile distilled water, and the suspension was then heated at 100ºC for twenty minutes. Accurately, 50-200 µl of the culture were placed in Eppendorf tube and started at -40 ºC until used.

DNA amplification for the virulent:

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany) using 25 µl of PCR mixture. The reaction mix invariably consisted of 5 µl of the bacterial lysate, 5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM MgCl2, 2 µl of 10mM dNTP mix 1 µl each of forward and reverse primer (10 pmol) and 1.25 U of Taq DNA polymerase made upto50 µl using sterile distilled water. The PCR cycling protocol was applied as following: An initial denaturation at 94°C for sixty seconds, followed by thirty five cycles of denaturation at 94°C for sixty seconds, annealing at 64°C for thirty seconds and extension at 72°C for thirty seconds, followed by a final extension at 72°C for seven minutes. Finally, 5 µl of each amplicon was electrophoresed in 1.5 % agrose gel (Sigma –USA, stained with ethidium bromide and visualized and captured on UV trans illuminator. A 100 bp DNA ladder was used as a marker for PCR products.

2.10. Statistical analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman *et al.,* (2003).

3. RESULTS

Total coliform count:

Results recorded in table (2) revealed that total coliform count (cfu/cm^2) of the tested samples obtained from abattoir A ranged from 3.40×10^5 to 8.10×10^7 with an average of $1.56 \times 10^7 \pm 5.46 \times 10^6$, but in case of abattoir B samples the counts were $3.80 \times 10^5$to $3.50 \times 10^8$with an average of $2.96 \times 10^7 \pm 1.75 \times 10^7$. Also for abattoir C, the mean coliform counts (cfu/cm2) were 2.60×10^5 to 3.60×10^7 with an average of $7.00 \times 10^6 \pm 2.32 \times 10^6$. In case of abattoir D, the mean coliform counts (cfu/cm2) ranged from 3.40×10^5 to 8.10×10^7 with an average of $1.16 \times 10^7 \pm 4.69 \times 10^6$.

From the results recorded in table (3) there is a highly significant differences ($P<0.01$) in the coliform count obtained from the 4 abattoir

Enterobacteriaceae count:

Results in table (4) revealed that total Enterobacteriaceae count (cfu/cm^2) of the tested samples obtained from abattoir A ranged from $3.30 \times 10^5$to $7.20 \times 10^8$with an average of $7.50 \times 10^7 \pm 4.08 \times 10^7 \pm$, but in case of abattoir B samples the counts were $3.30 \times 10^5$to $4.50 \times 10^8$with an average of $7.77 \times 10^7 \pm 3.44 \times 10$. Also for abattoir C, the mean Enterobacteriaceae counts (cfu/cm2) were $3.50 \times 10^5$to $8.90 \times 10^8$with an average $1.40 \times 10^8 \pm 6.54 \times 10^7$of. In case of abattoir D, the mean Enterobacteriaceae counts (cfu/cm2) ranged from $3.30 \times 10^5$to 6.80×10^8 with an average of $9.86 \times 10^7 \pm 4.91 \times 10^7$.

From results recorded in table (5) there is a highly significant differences ($P<0.01$) in the Enterobacteriaceae count obtained from the 4 abattoir.

Incidence of enterobacteriaceae:

In table (6), The incidence of Enterobacteriaceae isolated from samples of camel meat from the four abattoirs refer to Citrobacter diversus and freundii were (0% , 5%), (5% 15%), (0% , 0%) and (0%, 10%) but ,the incidence of the Enterobacter aerogenes, Enterobacter agglomerans, Enterobacter cloaa and Enterobacter hafniae) were (15% ,10% ,0% ,0%),(5% ,5%, 0%, 5%), (20%, 15% ,15% ,0%) respectively, and (5%, 5% , 20% ,5%) in A, B, C and D abattoirs respectively., Klebsilla ozaena and Klebsilla pneumoniae ratio were (15%, 25%),(10% ,20%),(0%, 35%) and (25%, 5%), Proteus mirabilis , Proteus rettgeri and Proteus vulgaris were (10%, 10% ,5%),(5% ,5%, 0%) (5%, 35% ,25%) and (10% ,20% ,20) but Serratia liquefaciens and serratia marcescens were (5%, 0%), (5% ,0%), (10%, 5%) and (20% ,5%) in A , B, C and D abattoirs respectively.

E. coli and its serotypes:

Incidence and serotyping of E. coli isolated from samples of camel meat in 4 abattoirs were represented in table (6), EHEC E coli strain were (O19 :H21 ,O111 :H4 , O121 :H7) with incidence (5%, 0%, 0%) in A and D abattoirs, (10% ,5% ,5%) in B and ,(5%, 0% ,5%) in C but EPEC E coli strains were (O44:H18 ,O86, O114:H4,O124,O171:H2) with their incidences (0%,5%,5%,5%,0%) isolated from A,(0%,0%,0% ,5%, 0%) isolated from B, the ratio was (10% ,5%, 0%, 5%,5%) in C and finally in D, the ratio was (19%, 0% ,5%, 5% ,0%), in addition to ETEC E coli strain were (0%) in A and D, and (5%) in B and C abattoirs, respectively.

Salmonellae and its serotypes:

The salmonellae isolated from the examined samples of camel meat from the different abattoirs were (S. Enteritidis, Typhimurium, Virchow, Heidberg, Kentucky and Infantis) with ratio (5%, 10%, 0%, 0%, 5%, 5%),(5%, 5%, 0%, 0%, 0%, 0%) ,(10%, 10%, 5%, 5%, 5% , 0%) and (5%, 5%, 0%, 0%,5%,5%) in A , B, C and D abattoirs ,respectively ,this showed in Table (8).

Occuranc of virulence gens of different isolated strains from examined samples of camel meat:

The virulence factors Stn, hilA and fim H were represented in S, Typhimurium,S, Enteritidis, S,KentuckyandS, Vichow. But S,infants had fimH, and S, Heidelberg had hila and fimH virulence factors showed in Table (9) and figure (1).

Table 1: Primer sequences of toxin producing genes in isolated *salmonella* strains.

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stn(F)	5′ CTTTGGTCGTAAAATAAGGCG ′3	260	Makino *et al.* (1999)
stn (R)	5′ TGCCCAAAGCAGAGAGATTC ′3		
hilA (F)	5′ CTGCCGCAGTGTTAAGGATA ′3	497	Guo *et al.* (2000)
hilA (R)	5′ CTGTCGCCTTAATCGCATGT ′3		
fimH (F)	5′ GGA TCC ATG AAA ATA TAC TC ′3	1008	Menghistu (2010)
fimH (R)	5′ AAG CTT TTA ATC ATA ATC GAC TC ′3		

Table 2: Statistical analytical results of total coliform count/(cfu/g) in the examined samples of camel meat from different abattoirs (n = 20).

abattoir	Positive samples						Positive samples
	No.	%	Min.	Max.	Mean	± S.E.M.	S.D
A	20	100	3.40×10^5	8.10×10^7	1.56×10^7	$\pm 5.46 \times 10^6$	2.44×10^7
B	20	100	3.80×10^5	3.50×10^8	2.96×10^7	$\pm 1.75 \times 10^7$	7.81×10^7
C	20	100	2.60×10^5	3.60×10^7	4.00×10^6	$\pm 2.32 \times 10^6$	1.04×10^7
D	20	100	3.40×10^5	8.10×10^7	1.16×10^7	$\pm 4.69 \times 10^6$	2.10×10^7

S.E. = standard error, S.D = Standard Deviation

Table 3: Analysis of variance (ANOVA) of total Coliform count/(cfu/g) in the examined samples of camel meat from different abattoirs (n=20)

	S. S	D.F	M.S	F	Sig
Between Groups	5.73×10^{15}	3	1.91×10^{15}	1. 055	(0.373)
Within Groups	1.38×10^{17}	76	1.81×10^{15}		
Total	1.43×10^{17}	79			

D.F = Degrees of freedom, S.S = Sum squares, M.S = Mean squares

++ = High significant differences (P<0.01)

Table 4: Statistical analytical results of total Enterobacteriaceae count/(cfu/g) in the examined samples of camel meat from different abattoirs (n = 20).

Abattoir	Positive samples						Positive samples
	No.	%	Min.	Max.	Mean	± S.E.M.	S.D
A	20	100	3.30×10^5	7.20×10^8	7.50×10^7	$\pm 4.08 \times 10^7$	1.83×10^8
B	20	100	3.30×10^5	4.50×10^8	9.77×10^7	$\pm 3.44 \times 10^7$	1.54×10^8
C	20	100	3.50×10^5	8.90×10^8	1.40×10^7	$\pm 6.54 \times 10^7$	2.93×10^8
D	20	100	3.30×10^5	6.80×10^8	7.86×10^7	$\pm 4.91 \times 10^7$	2.20×10^8

S.E.M. = standard error, S.D = Standard Deviation

Table 5: Analysis of variance (ANOVA) of total Enterobacteriaceae count/(cfu/g) in the examined samples of camel meat from different abattoirs (n=20).

Sum of Squares		df	Mean Square	F	Sig
Between Groups	5.37×10^{16}	3	1.79×10^{16}	0.375	)0.771(
Within Groups	3.63×10^{18}	76	4.77×10^{16}		
Total	3.68×10^{18}	79			

Table 6: Incidence of enterobacteriacea isolated from the examined samples of camel meat from different abattoirs.

Products	A		B		C		D	
Isolated bacteria	No.	%	No.	%	No.	%	No.	%
Citrobacterdiversus	-	-	1	5	-	-	-	-
Citrobacterfreundii	1	5	3	15	-	-	2	10
Enterobacteraerogenes	3	15	1	5	4	20	1	5
Enterobacteragglomerans	2	10	1	5	3	15	1	5
Enterobacter cloacae	-	-	-	-	3	15	4	20
Enterobacterhafniae	-	-	1	5	-	-	1	5
Klebsiellaozaenae	3	15	2	10	-	-	5	25
Klebsiellapneumoniae	5	25	4	20	7	35	1	5
Proteus mirabilis	2	10	1	5	1	5	2	10
Proteus rettgeri	2	10	1	5	7	35	4	20
Proteus vulgaris	1	5	-	-	5	25	4	20
Serratialiquefaciens	1	5	1	5	2	10	4	20
Serratiamarcescens	-	-	-	-	1	5	1	5

Table 7: Incidence and serotyping of E. coli isolated from the examined samples of camel meat from different abattoirs.

Product / *E.coli* Strains	A		B		C		D		Strain characteristic
	No.	%	No.	%	No.	%	No.	%	
O19:H21	1	5	2	10	1	5	1	5	EHEC
O44:H18	-	-	-	-	2	10	2	10	EPEC
O86	1	5	-	-	1	5	-	-	EPEC
O111:H4	-	-	1	5	-	-	-	-	EHEC
O114:H4	1	5	-	-	-	-	1	5	EPEC
O124	1	5	1	5	1	5	1	5	EPEC
O127:H6	-	-	1	5	1	5	-	-	ETEC
O121:H7			1	5	-		-		EHEC
O171:H2					1	5			EPEC
Total	4	20	6	30	7	35	5	25	

EPEC = Enteropathogenic E.coli ETEC = Enterotoxigenic E.coli

EHEC= Enterohaemorrhagic E.coli EIEC = Enteroinvasive E.coli

Table 8: Incidence and serotyping of Salmonellae isolated from the examined samples of camel meat from different abattoirs(n=20).

Products / Salmonella Strains	A		B		C		D		Group	Antigenic structure	
	No.	%	No.	%	No.	%	No.	%		O	H
S. Enteritidis	1	5	1	5	2	10	1	5	D1	1,9,12	g,m : -
S. Typhimurium	2	10	1	5	2	10	1	5	B	1,4,5,12	i : 1,2
S. Virchow	-	-	-	-	1	5	-	-	E1	3,10,15, 34	e,h : 1,6
S.Heidberg	-	-	-	-	1	5	-	-	E1	3,10,15, 34	e,h : 1,5
S.Kentucky	1	5	-	-	1	5	1	5	B	1,4,5,12	z_{10}: 1,2
S.Infantis	1	5	-	-	-	-	1	5	C1	6,7	r:1,2
Total	5	25	2	10	7	35	4	20			

Table 9: Occurrence of virulence genes of different Salmonella strains isolated from the examined samples of camel meat.

Virulence factors / *Salmonella* Serovars		*Stn*	*hilA*	*fimH*
S. Typhimurium		+	+	+
S. Enteritidis		+	+	+
S. Kentucky		+	+	+
S. infantis	-		-	+
S. Heidelberg		-	+	+
S. Virchow		+	+	+

stn: Enterotoxin gene, hilA: hyper-invasive locus gene, fimH: fimbrial gene

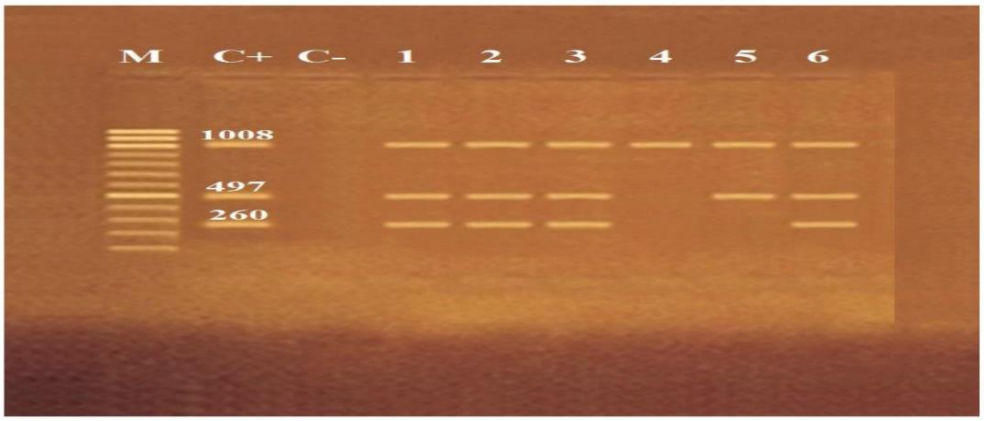

Fig.1. Agarose gel electrophoresis of multiplexPCR of stn (260 bp), hilA(497 bp) and fimH (1008 bp) virulence genes for characterization of Salmonella species.

Lane M: 100 bp ladder as molecular size DNA marker.

Lane C+: Control positive strainfor*stn, hilA* and *fimH* genes.

Lane C-: Control negative.

Lanes 1 (*S.Typhimurium*), 2 (*S.Enteritidis*), 3 (*S.Kentucky*) and 6 (*S.Virchow*): Positive strains for *stn, hilA* and *fimH* genes.

Lane 5(*S.Heidlberg*): Positive strainsfor*hilA* and *fimH* genes.

Lanes 4 (*S.infantis*): Positive strainsfor*fimH* gene.

4. DISCUSSION

Coliform count:

The presence of Coliforms on meat surface is common and has been isolated from different sites in variable numbers as reported by Mira (1989).

From results recorded in table (2) there is a highly significant differences ($P<0.01$) in the coliform count obtained from the 4 abattoir. This is due to variability of hygienic and manufacture practices done in each abattoir from the four abattoirs examined in this study.

Coliform organisms are mostly of fecal origin so we must avoid this fecal contamination through washing of the animal before entering the slaughterhouses, modernization of the abattoirs, education of the butchers about the bad effect of fecal contamination and sanitation and cleaning of the abattoir. The abattoirs in Egypt must be modernized and also the butchers must be educated about the hygienic practice to avoid contamination of meat from the skin during skinning and also great care must be taken during evisceration. The above results show that camel meat were highly contaminated with Coliform bacteria which suggest fecal contamination and points to potentially severe hazard (Eribo and Jay, 1985).

The occurrence of high numbers of Coliforms on the meat surfaces is important in reflecting the hygienic quality of meat and the test for Coliform bacilli is considered of much greater value in assessing its quality .The microbiological specification for meat purchased by U.S military and federal agencies was Coliform 10^2cfu /g*)* and also by comparing these with our results we found that all camel meat samples had Coliform count over 10^2cfu/g. The presence of Coliform group in meat has an epidemiological interest as some of its members are pathogenic, and may result in serious infection, and food poisoning ICMSF (1998). Thus, the total coliforms count may be used as aboard base indicating fecal contamination of meat. The coliform count must not exceed 10^2 cfu / g. meat (EOSQC 2062, 2005). This means that all our samples exceeds the permissible limits according to (*EOSQC* 2026, 2005).

Coliform count reflect inadequate sanitation during slaughtering, evisceration and handling of the carcasses, meat contact surfaces and butchers. However, the occurrence of large numbers of coliform in meat is highly undesirable and suggests mostly faecal contamination and sever hazard (Eribo and Jay, 1985).

From their original fecal, soil or plant environment, coliform can reach the food handlers hand, slaughterhouse environment, where they may be spread via equipment and utensils surfaces or by employees. The presence of coliform in meat depends upon the circumstances to which the food has been exposed and their presence in great number may rise the public health hazard. Although the bacterial count used in bacteriological examination to reflect the hygienic quality of meat, however, it is evident that the test for coliform bacilli is considered of much greater value in assessing its quality (National Academy of Sciences, 1985).

Enterobacteriaceae count:

It is clear from the previous results that the Enterobacteriaceae counts seem to be high and this draws our attention to the contamination from enteric sources so it can be used as proof for enteric contamination. The occurrence of high Enterobacteriaceae count indicated that there were poor sanitary conditions during slaughtering, handling and preparation as that was reported by Mira (1989).

The higher levels of *Enterobacteriaceae* counts after evisceration may be attributed to occasional rupture of viscera resulting in spread of gut contents onto the carcass (Viscera could be considered a potential source of contamination unless it was removed intact). Also, it was found that most of the contamination of camel carcasses occurred when the anal sphincter of rectum was separated from the carcass (Grau, 1986). The higher levels of *Enterobacteriaceae* counts after evisceration in abattoirs located in Menoufeia Province may be due to the slaughtering practices that were followed. The same butchers performed all of the slaughtering practices like removal of skin, evisceration and cutting using the same knives for all operations leading to spreading contamination. In contrast, in modern abattoirs, the evisceration process was carried out according to the production line with different butchers performing the different operations, thus neither the same person nor the same knife came in contact with the carcass during different operations.

Incidence of enterobacteriaceae:

Enterobacteriaceae contain many species, which have been reported to cause health hazard for the consumer, some other species are important from the economic point of view as they may cause spoilage and deterioration of meat and meat products (National academy of science, 1985).

Enterobacteriaceae group of bacteria is the most challenging bacterial contaminant to meat worldwide. *Salmonella, E. coli, Proteus* and *Klebsiella* species are the most predominant species in all food poisoning cases associated with some meat products (Al-Mutairi, 2011).

Our results in table (5) about the Enterobacteriaceae indicated that, the incidences of enterobacteriacae among examined camel samples cleared that, the higher enterobacteriacae isolates of a higher incidences observed in Proteus rettgeri, Klebsiellapnumoniae (35%), Nearly similar results were reported by (Wie, 2015). The frequency of isolation of *Citrobacter* species was lower in our results in camel meat samples, it present as *Citrobacterdiversus* in percentage of 5% in abbttoir B and *Citrobacterfreundii* in percentages of5,15,0 and 10 in abbttoirA,B,C and D . *Citrobacter* is a distinct group of human pathogens comprising three species: *Citrobacterfreundii*(biotypes a and b), *Citrobacteramalonaticus*, and *Citrobacterdiversus*. The most common sources of *citrobacter* isolates were urine, sputum, and soft tissue exudates. Members of this genus can cause neonatal meningitis and, perhaps, gastroenteritis in both children and adults. Although deep tissue infections due to *Citrobacter* have been reported only occasionally (Brenner *et al.,* 1993).

Enterobacter species was higher in camel meat as enterobacteraerogenes which present in percentage of 15,5,20 and 5, enterobacter agglomerani present in percentages of 10,5,15and 5 , Enterobacter cloaca present in percentage of 0,0,15and 20and Enterobacter hafniae present in percentages of 0,5,0and 5 in abattoir A, B, C and D , respectively. The Enterobacter species can be found in soil, water, sewage and intestinal tract of man and animals. This organisms are important in food as a potential health hazard and indicator organism for spoilage, also same strains of Enterobacter species have been implicated in acute and chronic diarrheal diseases and in severe cases of food poisoning (Bantwart, 1989).

Klebsiella species were isolated at high incidence from camel meat samples as well as , *klebsiella ozanae* was isolated in percentage of 15 ,10 ,0 and 25 from abattoirs A, B, C and

D, respectively, while klebsiella pneumonia was isolated in percentage of 25,20,35and 5 from abattoir A, B, C and D respectively. *Klebsiella ozanae and klebsiella aerogenes. Klebsiella* organisms have been implicated in chronic diarrhea disease, *Klebsiella pneumonia* was incriminated in cases of lobar pneumonia and other affections of respiratory tract and also it may cause meningitis, appendicitis, Pyaemia, cystitis (Mizuta et al., 1983).

Proteus species were isolated at higher incidence from camel meat samples in represented as *Proteus mirabilis* in percentages of 10,5,5, and 10 while *Proteus rettgeri* present in percentages of 10, 5, 35 , and 20 also *Proteus vulgaris* present in percentages of 5 ,0 ,5 and 20 in abattoirs A ,B ,C and D , respectively.

Proteus species are distributed in various foods, causing potential health hazards and spoilage. Certain species of proteus can cause enteric infection in human. Proteus species have been incriminated in causing summer diarrhea in infants, otitis, sinusitis and urinary tract infections (Varnam and Evans, 1991).The incidence of isolation of serratia species from camel meat was *Serratia liquefaciens* was isolated in percentages of 5,5,10, and 20 while *Serratia marcescens* was isolated in percentages of 0, 0, 5, and 5 in abattoirs A, B, C and D, respectively.

Rare reports have described disease resulting from infection with *Serratia plymuthica, Serratia liquefaciens, Serratia rubidaea, Serratia odorifera,* and *Serratia fonticola, Julie et al.,* (2009).

Meanwhile, our results about the incidences of enterobacteriaceae among examined samples collected from different localities concluded that, the enterobacteriaceae incidences in meat samples of camels depends upon the types of enterobacteriaceae and its isolates, regions and hygienic conditions found in the abattoir from the cleanliness and quality management of workers, equipment's, water used in the slaughtering processes in abattoir. It is also clear that carelessness during animal evisceration lead to intestinal rupture and releasing of intestinal contents which will lead to heavy contamination of different carcass parts by Enterobacteriaceae.

E. coli:

Our results in table (6) illustrated the incidences of different E. coli isolates of in different abattoirs .In abattoir A ,Enterohameorahgic E coli O19:H2 Enteropathogenic E coli O86, O114:H4 and O124 were isolated in the same incidence of 5%. In abattoir B, Enterohameorahgic E coli O111:H4 and O121:H7, Enteropathogenic E coli O124 and enterotoxigenic E coli O127 were isolated in the same incidence of 5% while Enterohameorahgic E coli O19:H12 was isolated in incidence of 10%. In abattoir C, Enterohameorahgic Ecoli O19:H21, Enteropathogenic E coli O86, O124and O171:H2 and enterotoxigenic E coli O127:H2 were isolated in the same incidence of 5% while, Enteropathogenic E coli O44:H18 was isolated in incidence of 10%. In abattoir D, Enterohameorahgic E coli O19:H21, Enteropathogenic E coli O124and O114:H4 were isolated in the same incidence of 5% while, Enteropathogenic E coli O44:H18 was isolated in incidence of 10%. Nearly similar results were reported by Saleh (2007).

The higher frequency of isolation of *E. coli* from camel meat samples indicated that there was bad pre slaughter management and poor sanitary conditions during slaughtering, handling and preparation. The carriage of *E. coli* in ruminants varies widely. Differences may arise because of methodology, season, cleanliness and transport associated stress prior to slaughter. The abattoir has been identified as

a major link in the transmission of *E. coli* to the food chain and cross contamination of the carcass with feces (Richards et al., 1998).

Our results on incidences of E. coli among examined samples collected from different abattoir of different localities at menofeia governorates:

This results concluded that, the E. coli incidences in meat samples of camels includes different isolates that includes EHEC, EPEC and ETEC. Their incidences depends upon the types of E.coli strains, regions and hygienic conditions found in the abattoir from the cleanliness and quality management of workers, equipments, water used in the slaughtering processes in abattoir.

These strains of E.coli is considered of fecal origin and its sources may be the slaughter line sources of E.coli were hide and fleece , workers' hands, fecal pellets, knife blades, intestinal contents and mastitic udder, *(*Tuteja *et al., 2003)* transport and/or lairaging of in abattoir ,penetrative stunning pistols and below hygienic standards water used in meat retail shops. In addation to this,*E. coli* was considered one of the important causes of febrile types of gastroenteritis transmitted by foods and considered the most common microorganism causing infant and children diarrheal cases. Buffaloes, cattle and sheep are the highest reservoir of *E. coli* infection to man (Taha 2002). *E. coli* O157:H7 is a pathogenic strain of *E. coli* known as enterohaemorrhaghic *E. coli* (EHEC). *E. coli* O157:H7 is a significant food-borne pathogen that has emerged in the past two decades. It colonizes the gastrointestinal tract and is associated with a range of symptoms, including watery or bloody diarrhoea, vomiting, haemorrhagic colitis and haemolyticuraemic syndrome, which are characterized by acute renal failure affecting mainly children and the immunocompromised (Griffin and Tauxe 1991).

Presence of E.coli in meat indicates a general lack of cleanness during slaughtering, evisceration, dressing, transportation and handling of meat. E.coli is most prominet fecal coliform. Hence, we might expect that there is a little difference between E.coli and fecal coliforms (ICMSF, 1996).

The detection of even low numbers of enteropathogenic E.coli in camel meat revealed a public health hazard as significant as demonstration of salmonellae in such meat , so we must educate the butchers and workers in the abattoir about the sources of contamination of E.coli to meat such as the remaining of fecal matter on the hide and fleece , also to avoid cross contamination through their hands. As well as the vehicle for transportation of meat must be clean and sanitized. Also adequately cleaned cutting equipment must be used to avoid contamination with E.coli.

Salmonellae:

Salmonella organisms contaminated animal carcasses via various routs including butcher's hands, knives, tables ,feces of animals slaughtered in abattoirs ,excessive handling of the carcasses, cross contamination, retail shop floors, lack of drainage, lack of dressing facilities, in Egypt, Salmonella was widely recognized as one of the most important causes of food poisoning outbreaks occurring as a result of consumption of contaminated meat and meat offal, *S.*enteritidis and *S. typhimurium* were the most frequent serotypes found in cases of human Salmonellosis. *Salmonella* was a common cause of enteric illness, which may be ranged from mild gastroenteritis to systemic illness such as septicemia and other longer term conditions, (FAO, 1992).

Our results of Salmonellae in table(7), cleared that, the incidences of different types of salmonella isolates among different examined samples of camel meat was higher in abattoir A, it was Salmonella Typhimurium in percentages of 10 and present in abattoirs B, C and D in percentage of 5. While for Salmonella Enteritidis the percentages were 5 in all abattoirs. Salmonella Virchow, Salmonella *Heidberg* and salmonellae *Kentucky* present only in abattoir C in percentages of 5% while Salmonella *Infantis* present in percentages of 5in abattoir A and D

Meanwhile, our results on incidence and serotyping of Salmonellae isolated from the examined samples of camel meat from different abattoirs indicated that, the higher incidences of Salmonellae differ according to the types of salmonellae, hygienic measures of abattoir and the locality of abattoir.

The contamination of meat by Salmonella organisms especially retailed meat in butchers shops may be originated from human carriers (workers) who handle and prepare the meat during cutting, deboning, slicing…etc. also it may be contaminated from infected carcasses in the slaughter house or from infected rodents which may be present in the butcher shops or slaughterhouse.

Salmonellosis is considered as one of the most important zoonotic diseases in which the main source of infection is food of animal origin and mortality due to *Salmonellosis* is relatively low and occurs only in very old individuals and infants *Salmonellosis* is characterized by clinical symptoms including gastroenteritis, typhoid and cholera like syndromes. Children suffering from Salmonellosis have sympyoms of dyspepsia, enterocolitis and typhoid fever, these symptoms are often accompanied by septicemia and bacteremia. S. typhimurium is the commonest Salmonella isolated from cases of food poisoning and represents about 50-60% of such (FAO*, 1992).*

In Egypt S. typhimurium was responsible for diarrheal cases by (1.6%), the etiological agent responsible for (27%) of acute gastroenteritis in infants in Alexandria is Salmonella organism and *S. typhimurium* was isolated from children suffering from acute gastroenteritis in Assiut (Ahmed and Shimamoto, 2014).

5. Conclusion

To avoid salmonella contamination of meat, we must avoid enteric contamination of meat, avoid human carriers of salmonellae to handle the meat and workers in the abattoir and butchers shops abeut personal hygiene as washing their hands after using the tollit. Meanwhile our results on the occurrence of virulence genes of different *Salmonella* strains isolated from the examined samples of camel meat cleared that, the virulence genes of different strains of isolated salmonellae differ according to the type of salmonellae.The Stn gene was observed in isolated strains of S. typhimurium, *S. Enteritidis, S. Kentucky, S. Heidelberg and S. Virchow.* The hilA gene was observed in isolated strains of S. typhimurium, *S. Enteritidis, S. Kentucky and S. Virchow.*The fimH gene was observed in isolated strains of S. typhimurium, S. Enteritidis, S. Kentucky, S. infants, S. Heidelberg and S. Virchow. While, our results on PCR cleared that, the virulence genes level of detection ranged from 260 to 1008 pb and at this level the genes ofstn, hilA and fimH genes, (S.Typhimurium), 2 (S.Enteritidis), 3 (S.Kentucky) and 6 (S.Virchow)**:** Positive strains for stn, hilA and fimH genes, (S.Heidlberg)**:** Positive strains forhilA and fimH genes, (S.infantis) Positive strains forfimH gene.

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