Phenotypic and genotypic studies on *Escherichia coli* strains isolated from food products of animal origin

Khafagy, A., A.;EL Shourbagy, M., A*.;Hanaa, M., F. and Samah, K. A.*****
*Bacteriology, Immunology and Mycology department- Faculty of Veterinary Medicine- Suez Canal University; * Anaerobics unit, animal health research institute, Dokki, Giza; ** Department of animal hygiene, zoonosis & animal behavior-Faculty of Veterinary Medicine-Suez Canal University and *** Faculty of Veterinary Medicine-Suez Canal University*

Abstract

Foodstuffs of animal origin may present hazards, due to bacterial contamination. This study was conducted to determine *Escherichia coli* in some types of animal source foods (raw milk, raw beef meat and raw poultry meat). Bacteriological examination of total 246 raw food samples of animal origin, raw milk, (105); beef meat (31) and poultry meat (110) were collected from different localities in Ismailia City showed that 64/246 (26.01%) of samples were infected with *E. coli*. Serological identification of (10) *E. coli* strains revealed that they were belonged to **O_{111} polyvalent 1**; **O_{44} polyvalent 2** (2 strains for each); **O_{78} polyvalent 4**; **O_{146} polyvalent 2**; **O_{26} polyvalent 1**; **O_{119} polyvalent 1**; **O_{86} polyvalent 1** and **O_{18} polyvalent 3** (1 strain for each). *E. coli* strains showed high susceptible rate to Ciprofloxacin (CIP), (100%) and high resistance (100%) to Ampicillin (AMP), Metronidazole (MTZ), Amoxicillin (AML), and Vancomycin (VAS). Four strains of the isolated *E. coli* were submitted to molecular studies for detection of the *eaeA* (protein intimin), *iutA* (encoding aerobactin) and *iss* (increased serum survival protein) genes, by using PCR technique.

Introduction

Foods and microorganisms have long and interesting association which developed longer before the beginning of recorded history ***(Dilbaghi and Sharma, 2007)***. Since its discovery in 1805, *E. coli* was considered a harmless, Gram-negative, motile, non-sporulating; rod shaped, facultative anaerobic bacterium and is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds. As it is present in high number in intestine, *E. coli* are often used as marker organisms. Detecting and counting of *E. coli* is used as a reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms in foods and water

which constitute a public health hazard ***(Kaper et al, 2004** and **Dilbaghi and Sharma, 2007)***. Pathogenic *E. coli* are classified into categories based on the production of virulence factors and on the clinical manifestations that they cause. In addition to the presence of ***E.coli*** denoting fecal pollution, the presence of virulence-related genes in *E. coli* strains refers to the pathogenicity of the isolates ***(Klie et al, 1997; Jayarao and Henning, 2001 and Holko et al, 2006)***. The protein intimin (encoded by the chromosomal gene *eae*), is responsible for the intimate attachment of the bacteria to intestinal epithelial cells ***(Law, 2000** and **Gyles, 2007)***. *iutA* encoding aerobactin system is one of serum survival genes, that are specific virulence markers associated with extraintestinal infection. The explanation of their occurrence could be the possibility of udder infection ***(Altalhi and Hassan, 2009)***. Isolates with the aerobactin system have a growth advantage in low-iron conditions ***(Montgomerie et al, 1984)*** and, in comparison to the other major specialized siderophore, enterobactin, aerobactin is more effective.

Considering the above mentioned data, the present study thus was planned to assess the bacteriological quality of some types of animal source foods by investigating ***Escherichia coli***, which may contaminate them, through the following topics:

1- Collection of raw milk, raw beef meat and raw poultry meat samples from different localities in Ismailia City.

2- Isolation of suspected *Escherichia coli* isolates which may be found in them.

3- Biochemical identification of the isolated ***Escherichia coli***.

4- Serological identification of some isolated *Escherichia coli* strains.

5- Antibiogram study of the isolated *Escherichia coli* strains.

6- Detection of the *eaeA* (protein intimin), *iutA* (encoding aerobactin) and *iss* (increased serum survival protein) gene factors that may be expressed in the examined *Escherichia coli* serovars.

Material and methods:

1- Isolation and identification of *E.coli* strains:

Escherichai coli were isolated from a total number of 246 raw food samples of animal origin, (105) raw milk samples, (31) beef meat samples and (110) poultry meat samples, by cultivation into 1% peptone water as pre-enrichment media, Brilliant Green Bile 2% broth as enrichment media, then on MacConkey's agar and by sub-cultivation on Sorbitol MacConkey's agar, sheep blood agar and Eosin methylene blue agar (EMB) and wrer identified by biochemical tests. Ten *E. coli* isolates were serologically identified by using slide agglutination test. Antibiotic

susceptibility of the isolated *E. coli* was performed, using standard plate technique as recommended by **Clinical laboratory Standard Institute (CLSI)**. Four strains of the serologically identified *E. coli* were submitted to molecular studies for detection of the *eaeA* (protein intimin), *iutA* (encoding aerobactin) and *iss* (increased serum survival protein) genes by using PCR technique.

2-Molecular studies on *E. coli* strain

2-1- Extraction of DNA, according to QIAamp DNA mini kit instructions

1- 20 µl QIAGEN protease were pipetted into the bottom of a 1.5 ml micro centrifuge tube, 200 µl of the bacterial broth culture and 200 µl buffer AL were added to the sample, then mixed by pulse vortexing for 15 seconds, then the mixture was incubated at 56˚C for 10 min. After incubation, the 1.5 ml micro centrifuge tubes were centrifugated to remove drops from the inside of the lid.

2- 200 µl ethanol (96%) were added to the sample, and mixed again by pulse vortexing for 15 seconds. After mixing, the 1.5 ml micro centrifuge tube was briefly centrifugated to remove drops from the inside of the lid.

3- The mixture from step 2 was carefully applied to the QIAamp mini spin column (in a 2ml collecting tube) without wetting the rim. The cap was closed, and centrifugated at 8000 rpm for 1 min. The QIAamp mini spin column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.

4- The QIAamp mini spin column was carefully opened and 500 ml buffer AW1was added without wetting the rim. The cap was closed, and centrifugated at 8000 rpm for 1 min. The QIAamp mini spin column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.

5- The QIAamp mini spin column was carefully opened and 500 ml buffer AW2 were added without wetting the rim. The cap was closed, and centrifugated at full speed for 3 minutes.

6- The QIAamp mini spin column was placed in a new 2 ml collection tube and the old collection tube was discarded with the filtrate. Centrifugation at full speed for 1 min was done.

7- The QIAamp mini spin column was placed in a clean 1.5 ml micro centrifuge tube, and the collection tube containing the filtrate was discarded. The QIAamp mini spin column was carefully opened and 100 µl buffer AE were added. The QIAamp mini spin column was incubated at room temperature (15-25˚C) for 1 minute, and then centrifugated at 8000 rpm for 1 minute.

2-2-Oligonucleotide primers sequences of *E. coli* genes:

2-3- Cycling conditions of the different primers during PCR of

the tested *E. coli* genes according to specific authors and Emerald Amp GT PCR mastermix (Takara) kit.

2-4- DNA Molecular weight marker

The ladder **(Gel Pilot 100 bp ladder** (cat. no. 239035), supplied from **QIAGEN (USA).**Number of bands: 6, Size range: 100-600 bp.**)**was mixed gently by pipetting up and down. 6 µl of the required ladder were directly loaded.

2-5- Agarose gel electrophoresis according to Sambrook *et al,* 1989, with modification

Electrophoresis grade agarose (2 g) was prepared in 100 ml TBE buffer in a sterile flask, it was heated in microwave to dissolve all granules with agitation, and allowed to cool at 70˚C, then 0.5µg/ml ethedium bromide was added and mixed thoroughly.

The warm agarose was poured directly in gel casting apparatus with desired comb in apposition and left at room temperature for polymerization.

The comb was then removed, and the electrophoresis tank was filled with TBE buffer. Ten to fifteen µl of each PCR product samples, negative control and positive control were loaded to the gel. The power supply was 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel was transferred to UV cabinet.

The gel was photographed by a gel documentation system and the data was analyzed through computer software.

Table (1): *Oligonucleotide primers sequences of E. coli genes:*

Primer	Sequence	Amplified product	References
eaeA	Forward/ GACCCGGCACAAGCATAAGC	384 bp	**Wen-jie JIN *et al.*, 2008**
	Reverse/ CCACCTGCAGCAACAAGAGG		
Iss	Forward/ ATGTTATTTTCTGCCGCTCTG	266 bp	**Yaguchi *et al.*, 2007**
	Reverse/CTATTGTGAGCAATATACCC		
iutA	Forward/ GGCTGGACATGGGAACTGG	300 bp	
	Reverse/CGTCGGGAACGGGTAGAATCG		

Table (2) *Cycling conditions of the different primers of the E. coli genes*

Genes	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
eaeA	94˚C 5 min.	94˚C 45 sec.	54˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.
Iss	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	35	72˚C 10 min.
iutA	94˚C 5 min.	94˚C 30 sec.	63˚C 30 sec.	72˚C 30 sec.	35	72˚C 10 min.

Results:

*Biochemical examination revealed identification of 64 *E. coli* strains at percentage of 26.01% of the examined samples (27 strains, at percentage of 25.71% of the examined milk samples, 8 strains, at percentage of 25.80% of the examined beef meat samples and 29 strains, at percentage of 26.36% of the examined poultry meat samples).

*Serological identification of (10) *E. coli* isolated from the examined samples revealed that they were belonged to **O_{111} polyvalent 1**; **O_{44} polyvalent 2** (2 strains for each); **O_{78} polyvalent 4**; **O_{146} polyvalent 2**; **O_{26} polyvalent 1**; **O_{119} polyvalent 1**; **O_{86} polyvalent 1** and **O_{18} polyvalent 3** (1 strain for each).

*The antimicrobial sensitivity of the isolated *E. coli* showed high susceptible rate to Ciprofloxacin (CIP), (100%), followed by Cefoperazone (CFP), (75%); Enrofloxacin (ENR), (73.44%); Cefotaxime (CTX), (70.31%); Streptomycin (S), (48.44%) and Colistin sulphate (CT), (45.31%). On the other hand, all strains (100%) were resistant to Ampicillin (AMP), Metronidazole (MTZ), Amoxicillin (AML) and Vancomycin (VAS).

*The molecular studied resulted in:

- The protein intimin (encoded by *eae* gene) was found in *E. coli* serovars ; **O_{18} polyvalent 3**; **O_{111} polyvalent** 1and **O_{44} polyvalent 2**, with a size of 384 base pairs, while the examined gene was not found in ***E. coli*** serovar, **O_{146} polyvalent 2**.
- The aerobactin *iutA* gene was present in the four examined *E. coli* serovars , **(O_{18} polyvalent 3; O_{111} polyvalent 1; O_{44} polyvalent 2,** and **O_{146} polyvalent 2**), with a size of 300 base pairs.
- ***coli*, O_{146} polyvalent 2**, did not have the increased serum survival *iss* gene, while the other 3 serovars (**O_{18} polyvalent 3; O_{111} polyvalent 1 and O_{44} polyvalent 2**) had it, with a size of 266 base pairs.

Table (3): *Results of biochemical identification and frequency of distrebution of E. coli among the examined food samples*

Types of samples	No. of samples	No. of isolated *E. coli*	%
Raw milk	105	27	25.71
Raw beef meat	31	8	25.80
Raw Poultry meat	110	29	26.36
Total	246	64	26.01

Table (4) *Results of serological identification of the examined E. coli strains (10 strains)*

Monovalent	Polyvalent	No.
O_{111}	polyvalent 1	2 strains
O_{44}	polyvalent 2	2 strains
O_{78}	polyvalent 4	1 strain
O_{146}	polyvalent 2	1 strain
O_{26}	polyvalent 1	1 strain
O_{119}	polyvalent 1	1 strain
O_{86}	Polyvalent 1	1 strain
O_{18}	polyvalent 3	1 strain

Table (5) *Antimicrobial sensitivity test of the isolated Escherichia coli serovars from the examined samples*

Antimicrobial groups			Antimicrobial agent	Sympol	Concentation	Antibiotic sensitivity pattern					
						Sensitive		Intermediate		Resistant	
						No	%	No	%	No	%
		Penicillins Aminopenicillins (Pen A)	Amoxicillin	(AML)	10 ug	0	0	0	0	64	100
			Ampicillin	(AMP)	10 ug	0	0	0	0	64	100
Beta lactams		3rd generation Cephalosporins (C_3G)	Cefoperazone	(CFP)	75 ug	48	75	16	25	0	0
			Cefotaxime	(CTX)	30 ug	45	70.31	19	29.69	0	0
		Aminoglycosides	Streptomycin	(S)	10 ug	31	48.44	33	51.56	0	0
		Poly peptides	Colistin sulphate	(CT)	10 ug	29	45.31	35	54.69	0	0
		Glycopeptides	Vancomycin	(VAS)	30 ug	0	0	0	0	64	100
Quinolones		Fluoroquinolones	Ciprofloxacin	(CTP)	5 ug	64	100	0	0	0	0
			Enrofloxacine	(EnR)	5 ug	47	73.44	17	26.56	0	0
		Others	Metronidazole	(MTZ)	5 ug	0	0	0	0	64	100

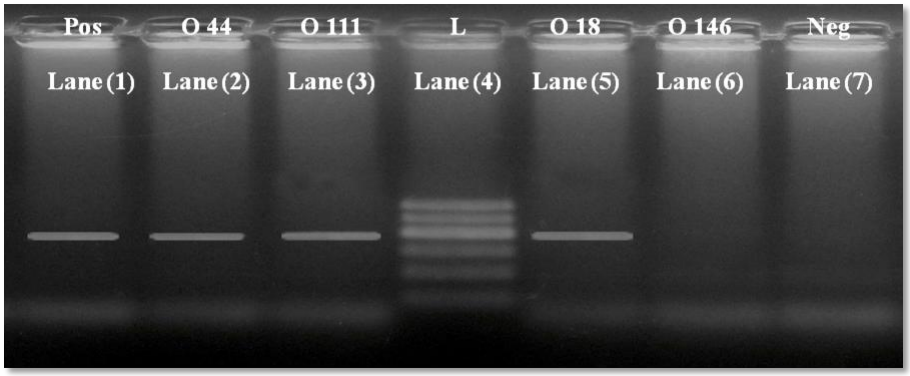

Figure (1) *Agarose gel electrophoresis of amplified eaeA gene PCR product (384bp)*
Lane1: Positive control for *eaeA* gene.
Lane 2 ,3 &:5 : Positive strains for *eaeA* gene (384 bp).
Lane 4: One step ladder (600bp).
Lane 6: Negative strains for *eaeA* gene.
Lane7: Negative control.

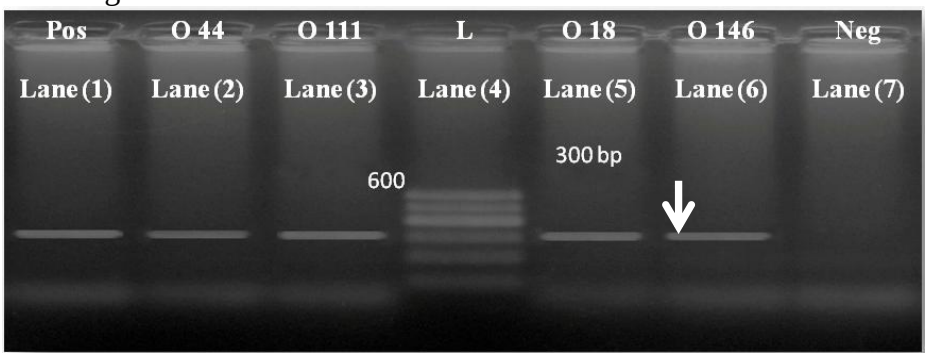

Figure (2) *Agarose gel electrophoresis of amplified iutA gene PCR product (300 bp)*
Lane1: Positive control for *iutA* gene.
Lane 2 ,3, 5 and 6 : Positive strains for *iutA* gene (300 bp)
Lane 4: 100 bp ladder (100-600bp).
Lane7: Negative control for *iutA* gene.

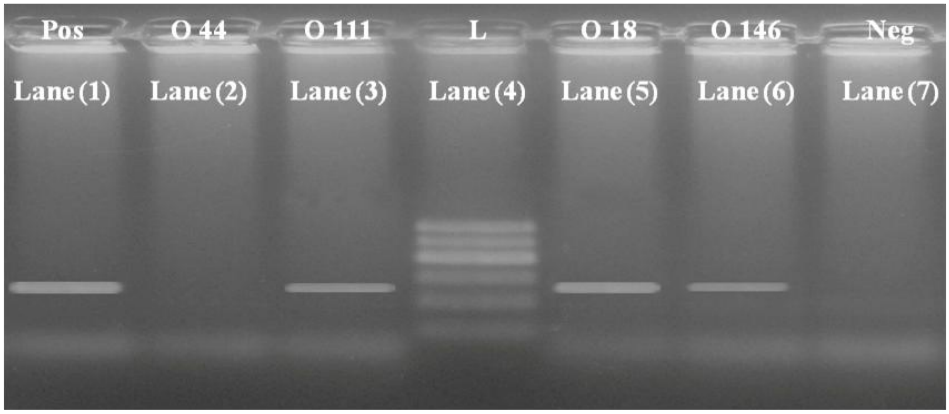

Figure (3) *Agarose gel electrophoresis of amplified iss gene PCR product (266 bp)*
Lane1: Positive control for *iss* gene.
Lane2: Negative strain for *iss* gene.
Lane 3, 5 and 6 : Positive strains for *iss* gene (266 bp)
Lane 4:100 bp ladder (100-600bp).
Lane7: Negative control

Discussion

Out of 105 raw milk samples, ***Escherichia coli*** could be detected in 27 samples, comprising 25.71% of the examined raw milk samples. Corresponding results were obtained by *El-Jendy, (2004),* who isolated ***E.*** 24% of the examined samples. Higher values were reported by *Abd-Allah (2002),* (66%) from raw market samples & (86%) from raw bulk milk samples. *Sudershan & Ashwani (1996),* recovered *E. coli* at lower level (10.19%) of 108 raw milk samples, collected from Hisar, India.

The presumed route of ***E. coli*** contamination of raw milk is via fecal contamination during milking *(Hussein & Sakuma, 2005).* However, direct excretion of the organisms from the infected udder has also been reported *(Lira et al., 2004).* Moreover, ***E. coli*** is regarded as an indicator of poor hygiene and sanitary practices during milking and further handling, ***(Altalhi & Hassan, 2009)***.

For the examined raw meat samples, 8 isolates of ***Escherichia coli***, at percentage of (25.80%) of the examined samples. Nearly similar result was given by *Mona (2002),* (24%). Higher values were obtained *by* ***Elwi (1994)***, (93.3%). Lowe records were given *Neveen (2002),* (10%). Opposite result was stated by *Khalafalla (1996),* who determined 30 samples of raw meats (10 each of brisket meat, chuck meat and cubes from hind quarter) and ***E.coli*** were failed to be detected from any of the examined samples.

E.coli and ***Salmonella*** species are two of bacterial genera that commonly infect meat while it is being processed, cut, packaged, transported, sold and handled. These organisms spoiling meat may infect the animal either while it is live "endogenous disease" or may contaminate the meat after its slaughter "exogenous disease" ***(Lawrie & Ledward, 2006).***

Twenty nine isolates of ***Escherichia coli*** were obtained from the examined raw poultry meat samples, at percentage of 29.59% of the examined samples. Corresponding results were mentioned by ***Mona (2002),*** **(30%)** Higher values were shown by ***Rofiel (1999)***, (40%); Lower result was given by ***Ghada (1997)***, (20%).

Serological identification of (10) representative *E. coli* strains, isolated from the examined samples, raw milk, raw beef meat and raw poultry meat, revealed that they were belonged to O_{111} polyvalent 1, O_{44} polyvalent 2 (2 strains for each), O_{78} polyvalent 4, O_{146} polyvalent 2, O_{26} polyvalent 1, O_{119} polyvalent 1, O_{86} polyvalent 1 and O_{18} polyvalent 3 (1 strain for each). **Noha (2008)** isolated *Escherichia coli* from slaughtered and apparently healthy chicken, which serologically identified as O_{78}, O_1, O_2, O_8, O_{27} O_{119}, O_{126}, O_{125}, O_{26}, O_{87}, O_{128} and O_{146}.

Enteropathogenic *Escherichia coli* (EPEC) traditionally has been reserved for the classic serovars: O_{26}, O_{86}, O_{111}, O_{114}, O_{119}, O_{125}, O_{128}, O_{142} and O_{158} and are a special group organisms associated with out-breaks of children diarrhea ***(Wolfang et al, 1992)***.

All strains (100%) showed resistance to Ampicillin (AMP), Amoxicillin (AML), (both are related to Beta lactams, Aminopenicillins); Vancomycin (VAS), (Glycopeptides) and Metronidazole (MTZ).

Some of this bacterial resistance related with R-factor that lead to trance multiple resistance of antibiotic at some time and that happens mostly in bacteria that cause diarrhea ***(Smith et al, 1973; Ryder et al, 1980 and Sur et al, 2003).***

The protein intimin (encoded by *eae* gene) was expressed in *E. coli* serovars; O_{18} polyvalent 3; O_{111} polyvalent 1and O_{44} polyvalent 2, with a size of 384 base pairs, while the examined gene was not expressed in *E. coli* serovar O_{146} polyvalent 2. ***Jelacic et al (2003)*** analyzed, by polymerase chain reaction, 82 Shiga toxin–producing *Escherichia coli* (STEC) isolates and found that all *E. coli* O157:H7 contained *eae*.

The aerobactin *iutA* gene was expressed in the four examined *E. coli* serovars, (O_{18} polyvalent 3; O_{111} polyvalent 1; O_{44} polyvalent 2, and O_{146} polyvalent 2), with a size of 300 base pairs. ***Altalhi and Hassan (2009)*** screened *E. coli* strains, isolated from raw milk sources, for markers of extraintestinal pathogenic *E. coli*

(ExPEC) using PCR assays and suggested that *iutA* (11 strains) was one of the most frequent virulence markers. Systems to meet the bacterial need for iron during infection is one of factors that are probably important for the establishment of Enterohaemorrhagic *Escherichia coli* (EHEC) in the gut and add to the bacterial virulence ***(Welinder-Olsson and Kaijser, 2005).***

E. coli O_{146} polyvalent 2, did not react the increased serum survival gene *iss* positively with *iss* gene, while the other 3 serovars (O_{18} polyvalent 3; O_{111} polyvalent 1 and O_{44} polyvalent 2) expressed it, with a size of 266 base pairs. examined the prevalence of the three *iss* types among 487 *E. coli* isolates and found that the *iss* type 3 geneS was found to occur at a high frequency among extraintestinal pathogenic *Escherichia coli* (ExPEC) isolates, irrespective of the host source and reported that the increased serum survival gene *iss* has long been recognized for its role in extraintestinal pathogenic *Escherichia coli* (ExPEC) virulence.

The findings of this study revealed that some raw foods of animal origin sold to the public in some areas in Ismailia City were contaminated with *E.coli*. The possible sources of these contaminants are due to the unhygienic manner of handling .This implies that these foods are viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards. It is believed that cooking processes and hygiene could greatly reduce the microbial load to harmless level ***(Agnes, 1995).***

References

Abd-Allah, M.I.M.I. (2002): Hygienic quality of concentrated and raw milks sold in Damietta Governorate. Ph. D. of Vet. Med. Sci. thesis (Hygiene of milk, milk products, fats & oils and eggs). Department of Food Control, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Agnes, C.H. (1995): Food Microbial Journal 16:226-280.

Altalhi, D. A. and Hassan A. S. (2009): Bacterial quality of raw milk investigated by *Escherichia coli* and isolates analysis for specific virulence-gene markers Food Control; 20: 913–917.

Dilbaghi, N. and Sharma, S. (2007): Food and industrial microbiology. "Food spoilage, food infections and intoxications caused by microorganisms and methods for their detection". Introduction, P. (1-3).

El. Jendy, I.M.S.I.A. (2004): Studies on ***Enterobacteriaceae*** in milk and some dairy products. M. vet. Med. Sci. thesis (Hygiene of Milk, Milk products, fat & oils and eggs). Department of Food Control, Zagazig University, Egypt.

Elwi, E.M. (1994): Sanitary improvement of meat meals in governmental hospitals in Assiut

City. Ph. D. Vet. Sci. thesis (Meat hygiene), Department of food control, Faculty of veterinary medicine, Assiut University, Egypt.

Ghada, M.I. (1997): Microbiological and sensory evaluation of fresh broiler chicken carcass. M. Vet. Sci. thesis (Meat hygiene), Department of food hygiene, Faculty of veterinary medicine, Alex. University, Egypt.

Gyles, C.L. (2007): Shiga toxin-producing *Escherichia coli*: an overview. Journal of Anim Science; 85: E45–62.

Holko, I.; Bisova, T.; Holkova, Z. and Kmet, V. (2006): Virulence markers of *Escherichia coli* strains isolated from traditional cheeses made from unpasteurised sheep milk in Slovakia. Food Control, 17, 393–396. Wochenschrift; 110: 337–341.

Hussein, H.S. and Sakuma, T. (2005): Prevalence of shiga toxin-producing ***Escherichia coli*** in dairy cattle and their products. Journal of Dairy Science; 88: 450–65.

Jayarao, B.M. and Henning, D.R. (2001): Prevalence of food borne pathogens in bulk tank milk. Journal of Dairy Science, 84(10): 2157- 2162.

Jelacic, K.J.; Damrow, T. ; Chen, G.S.; Jelacic, S.; Bielaszewska, M.; Ciol, M., Carvalho, H.M.; Melton-Celsa, A. R.; O'Brien, A.D. and Tarr, P.I. (2003): Shiga Toxin–Producing *Escherichia coli* in Montana: Bacterial Genotypes and Clinical Profiles. J Infect Dis.; 188 (5): 719-729.

Kaper, J. B., Nataro, J. P., and Mobley, H. L.T (2004): Pathogenic *Escherichia coli*. Natural Reviews Microbiology; 2: 123–40.

Khalafalla, F.A. (1996): Microbial evaluation of raw meats, meat products and non-meat ingredients. Beni-Suef Vet. Med. Res. VI, 2: 141.

Klie, H.; Timm, M.; Richter, H.; Gallien, P.; Perlberg, K.W. and Steinruck, H. (1997): Detection and occurrence of verotoxin-forming and/or Shigatoxin producing *Escherichia coli* (VTEC and/or STEC) in milk. Berliner und Munchener Tierarztliche Wochenschrift; 110: 337–341.

Law, D. (2000): Virulence factors of *Escherichia coli* $\mathbf{O_{157}}$ and other Shiga toxin-producing *E. coli*. Journal of Applied Microbiology; 88:729–45. **Lawrie, R.A. & Ledward, D.A. (2006):** The Preservation Effect of smoke on Meat. *Meat science*. Cambridge, Woodhead Publication.

Lira, W.M.; Macedo, C. and Marin, J.M. (2004): The incidence of Shiga toxin-producing ***Escherichia coli*** in cattle with mastitis in Brazil. Journal of Applied Microbiology; 97: 861–6.

Mona, M.I. (2002): Bacteriological studies on food poisoning micro-organisms present in hospitals food of animal origin and their handlers. Ph.D.Vet. Sci. thesis (Bacteriology, Immunology & Mycology), Faculty of veterinary medicine, Suez Canal University, Egypt.

Montgomerie, J.Z.; Bindereif, A.; Neilands, J.B.; Kalmanson, G.M. and Guze, L.B. (1984): Association of hydroxamate siderophore (aerobactin) with *Escherichia coli* isolated from patients with bacteraemia. Infect Immun; 46: 835_ 8.

Neveen, H.E.A. (2002): Microbiological investigations of meat serving establishments . Ph. D. Vet. Med. Sci. thesis (Meat, fish & poultry Hygiene), Departement of food control, Faculty of veterinary medicine, zagazig University, Egypt.

Noha, A.H. (1995): ***Samonellae*** in meat and poultry meat products using immune fluorescent technique. M. Vet. Sci. thesis (Meat hygiene), Faculty of veterinary medicine, Cairo University, Egypt.

Rofiel, B.Y. (1999): Aspects on organoleptic and microbiological changes of frozen poultry. M. Vet. Med. Sci. thesis (Meat Hygiene), Faculty of veterinary medicine, Zagazig University, Egypt.

Ryder, R.W.; Blake, P.A.; Murlin, A.C.; Carter, G.P.; Pollard,R.A.; Merson,N.H.; Allen, S.D. and Brenner, D.J. (1980): Increase in antibiotic resistance among isolates of *Salmonella* in United States, Journal of Infectious Diseases; 142(4): 485-490.

Sambrook, J.; Fritsch, E.F. and Maniatis, T. (1989): Molecular coloning: A laboratory manual, Vol 1, 2nd Edition. Cold spring HarborLaboratotry press, New York.

Smith, H.R.; Grindly, N.D.; Hampheryes, G.O. and Anderson, K.O. (1973): *Salmonella* and *Campylobacter*. J. Bact. 115:623.

Sudershan, K. and Ashwani, K. (1996): Occurrence of some bacterial pathogens of public health significance in bovine raw milk. Haryana Vetrinarian; 35; 31-34. Dairy Science Abst.; 60(4):281,(1998) .

Sur, D.; Niyogi, S.; Sur, S.; Tackeda, Y. and Nair, G. (2003): Multidrug resistant *Shigella dysenteriae* type1 fore run-ners of anew epidemic strain in eastern India. Emerg. Infect. Dis.; 9(3): 404-405.

Welinder-Olsson, C. and KAIJSER, B. **(2005):** Enterohemorrhagic *Escherichia coli* (EHEC) Scandinavian Journal of Infectious Diseases; 37: 405_ 416.

Wen-jie J.;Zhi-ming Z.; Yong-zhi Z.;Ai-jian Q.; Hong-xia S.; Yue-long L.; Jiao W. and Qian-qian W. (2008): Distribution of Virulence-Associated Genes of Avian Pathogenic *Escherichia coli* Isolates in China. Agricultural Sciences in China, 7(12): 1511-1515.

Wolfgang, K.J.; Hilda, P.W.; Bernard, D.A. and Catherine, M.W. (1992): Opportunistic *Enterobacteriaceae*. In zinsser microbiology 20th Edition. Prentic-Hall International Inc. USA, New York.

Yaguchi, K.; Ogitani, T.; Osawa, R.; Kawano, M.; Kokumai, N.; Kaneshige, T.; Noro, T.; Masubuchi, K. and Shimizu, Y. (2007): Virulence factors of avian pathogenic *Escherichia coli* strains isolated from chickens with colisepticemia in Japan. Avian Dis. 2007 Sep;51(3):656-62.

دراسات ظاهرية وجينية عن ميكروب إيشريشيا كولاى المعزول من المنتجات الغذائية من أصل حيوانى

أحمد أحمد رفعت خفاجى- ممدوح عبد العظيم الشوربجى*
هناء محمد فاضل - سماح كمال أحمد سلامة*****
قسم البكتيريا والمناعة و الفطريات- كلية الطب البيطرى- جامعة قناة السويس
* وحدة اللاهوائيات- معهد بحوث صحة الحيوان- الدقى، الجيزة.
** قسم صحة الحيوان والأمراض المشتركة وسلوكيات الحيوان.
***طبيبة بيطرية بكلية الطب البيطرى.

تمثل الأغذية ذات الأصل الحيوانى خطورة على الصحة العامة نتيجة ما قد تحمله من ملوثات بكتيرية وتعتبر الاختبارات الميكروبيولوجية وسيلة للحكم على مدى أمان وجودة هذه الأغذية. الفحص البكتيرى لعدد ٢٤٦ عينة غذاء من أصل حيوانى (١٠٥) عينة ألبان خام، (٣١) عينة لحوم عجالى و(١١٠)عينة لحوم دواجن ، أسفر عن عزل وتصنيف (بيوكيميائياً) ٦٤ بكتريا إيشريشيا كولاى ، بنسبة ٢٦,٠١% من مجموع العدد الكلى للعينات التى تم فحصها (٢٧ عترة بنسبة ٢٥,٧١% من مجموع عينات الألبان ،٨ عترات بنسبة ٢٥,٨٠% من مجموع عينات اللحوم العجالى ،٢٩ عترة بنسبة ٢٦,٣٦% من مجموع عينات لحوم دواجن). تم تحديد الأنواع المصلية لعشر عترات من الإيشريشيا كولاى المعزولة من العينات المختبرة.تم عمل اختبار الحساسية حيث أظهرت حساسة بنسبة ١٠٠% نحوسيبروفلوكساسين (٥ميكروجرام)، بينما أظهرت مقاومة بنسبة ١٠٠% ضد أمبسللين (١٠ميكروجرام) ، ميترونيدازول (٥ميكروجرام) ، أموكساسللين (١٠ميكروجرام) ، فانكوميسين (٣٠ميكروجرام). تم عمل اختبارات جزيئية على أربعة أنوع مصلية للإيشريشيا كولاى للبحث عن ثلاثة من عومل الضراوة التى تزيد من خطورة الميكروب، وهى جينات الإنتيمين، الإيروباكتين، والإسس، حيث وجد جين الإنتيمين فى ثلاثة أنواع مصلية من الأربعة، ووجد جين الإيروباكتين فى الأربعة أنواع، ووجد جين الإسس فى ثلاثة فقط منهم.