ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

Prevalence and biocontrol of shiga toxin producing *Escherichia coli* in some beef products

A thesis submitted to faculty of Veterinary Medicine University of Sadat City for the degree of Ph. D.

(Meat Hygiene & Control)

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2015

University of Sadat city

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Approval sheet

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ACKNOWLEDGEMENT

First of all, prayerful thanks to my Glorious Allah who gives me every thing I have.

I wish to express my grateful thanks and sincere gratitude to my supervisor ***prof.Dr. Abdel-Rahman M. El-Bagory***, Professor of Milk Hygiene and Head of Food Hygiene & Control Department, Fac. of Vet. Med., Sadat City University for his continuous supervision, helpful advice, guidance and his interest which have made possible the completion of this work.

I wish to express my deepest appreciation and sincere gratitude to my supervisor ***Prof.Dr. Abo-Baker M. Edris***, Professor of Meat Hygiene, Faculty of Vet. Med., Moshtohor, Benha University, for his endless help, continuous supervision, extreme valuable advices, great efforts, discussions and guidance throughout this study.

I wish to express my deepest appreciation and sincere gratitude to ***Prof.Dr. Mohamed A. Hassan***, Professor of Meat Hygiene, Faculty of Vet. Med., Moshtohor, Benha University, for his endless help in completing the part of biocontrol of *E.coli* in minced meat products.

Grareful thanks to ***Dr. Ahmed Elsify***, Lecturer of Infectious Diseases, Faculty of Vet. Med., Sadat City University, for his endless help, extreme valuable advices, great efforts, discussions and guidance throughout this study.

It is a great pleasure for me to express my thanks to the staff members of the Food Hygiene & Control Department, Fac. of Vet. Med., Sadat city University, for providing facilities, and continuous assistance during this work.

Finally, I am very grateful to every body else who contributed in any way to this work.

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List of abbreviations

APHA	American public health association
DAEC	Diffuse adhering *E.coli*
E.coli	*Escherichia coli*
eae	Intimine gene

EAEC	Enteroaggregative *E.coli*
EHEC	Enterohemorrhagic *E.coli*
EOSQ	Egyptian organization for standardization and quality control
EPEC	Enteropathogenic *E.coli*
ETEC	Enterotoxigenic *E.coli*
ExPEC	Extraintestinal pathogenic *E.coli*
HACCP	Hazard analysis and critical control point
HC	haemorrhagic colitis
hlyA	Hemolysin gene
HUS	haemolytic uraemic syndrome

ICMSF	International commission on microbiological specification for food
PCR	Polymerase chain reactions
STEC	Shiga toxin producing *E.coli*
Stx1	Shiga toxin type 1
Stx2	Shiga toxin type 2
TTP	thrombotic thrombocytopenic purpura
USDA	United States Department of Agriculture
VCA	Vero Cytotoxicity Assay
VTEC	verotoxin-producing *E. coli*

1-Introduction

In Egypt, meat products such as minced meat, kofta, sausage, beef burger and luncheon are gaining popularity because they represent quick easily prepared meat meals and solve the problem of the shortage in fresh meat of high price which is not within the reach of large numbers of families with limited income.

Contamination of such meat products with some foodborne microorganisms as *E. coli* during further processing make us in need to use rapid and accurate methods for their detection and to establish appropriate control measures to get red of such organisms.

As the traditional methods for detecting and identifying foodborne pathogenic microorganisms require presumptive tests followed by confirmative ones which are time consuming, so several rapid methods have been developed for the detection of foodborne microorganisms as Enzyme–linked Immunosorbant Assay (ELISA) and Polymerase Chain Reaction (PCR).

Acutally, PCR allows several millions fold amplification of target DNA from as little as one copy in 2 to 3 hours and considered as a highly sensitive, specific and rapid method for detection of foodborne microorganisms as *E.coli*, that substitute the biochemical and serological characterization of the pathogen. In addition, PCR can be applied on fixed tissues (frozen or formalin fixed) reducing the potential dangers involved in transport and handling of specimens with live virulent pathogens ***(Reinoso et al., 2004)***. Moreover, Polymerase Chain Reaction (PCR) technology allows simultaneous amplification and detection of specific gene targets, that providing immediate identification of pathogenic *E.coli*.

In general , *E. coli* is a major component of the normal intestinal flora of human and other mammals which are usually harmless to the host and only cause diseases in immunocompromised hosts or when the gastrointestinal barriers are breached. However, some specific *E.coli* strains represent primary pathogens with enhanced potential to cause disease after acquiring specific virulence attributes. These virulence attributes are normally encoded on genetic elements that can be exchanged between different strains or on those elements once having been mobile but later becoming fixed into the genome. Specific combinations of virulence factors form different pathotypes of *E. coli* based on the various human diseases. ***(Li et al., 2005)***

Pathogenic *E. coli* have been broadly classified into two major categories; the diarrheagenic *E. coli* and the extraintestinal pathogenic *E. coli*. Among the diarrheagenic *E. coli*, there are currently six categories including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusively adherent *E. coli* (DAEC) and enterohemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC).***(Xiaodong., 2010).***

Shiga toxin–producing *E. coli* (STEC), also known as verotoxin-producing *E. coli* (VTEC) or enterohaemorrhagic *E. coli* (EHEC), have been known as a group of highly pathogenic *E.coli* strains producing one or more Shiga toxins (***Monaghan et al., 2011***). The term verocytoxin producing *E. coli* was derived form observation of strains producing a toxin with a profound and irreversible cytopathic effect on Vero cells "African green monkey kidney" **(Konowalchuk et al., 1977).**

STEC represent a hazardous public health problem worldwide causing various human gastrointestinal tract diseases, including watery or bloody diarrhea and might develop a life-threatening diseases, such as haemorrhagic colitis (HC), Thrombotic Thrombocytopenic Purpura (TTP) and Haemolytic Uraemic Syndrome (HUS). The later is characterized by thrombocytopenia, microangiopathic haemolytic anaemia and acute renal failure (***Pennington., 2010***).

STEC strains produce two powerful phage-encoded cytotoxins causing tissue damage in humans and animals, called Shiga toxins or verotoxins (Stx1/VT1 and Stx2/VT2), which are the common feature and main virulence factors of STEC and are directly correlated with human pathogenicity (***Lindgren et al., 1993***). Stx2 is the most powerful toxin, and the toxin producing strains are usually associated with more severe infections (***Muniesa et al., 2004 and Gyles, 2007***). In addition, some STEC strains can tightly attach and form attaching and effacing lesions to intestinal epithelial cells through an adhesin called intimin, which is encoded by the *eae* gene.

As Shiga toxin–producing *E. coli* (STEC) are considered public health hazards , various approaches for minimization and controlling of STEC in meat product were tried . Natural preservatives as nisin alone or combined with other chemicals to be effective against *E.coli* were used for biocontrol of *E.coli* by many authors **(Catherine and Gregory, 1995** and **Eleiwa- Nesereen, 2003)**.

Nisin " bacteriocin" is an antimicrobial peptide produced by some strains of *Lactococcus lactis* **(Bender and Bender, 1995)** and is used in meat technology as a chemical preservative where it has a powerful inhibitory effect against Gram-positive bacteria, but probably has not the same effect on Gram-negative ones as

E.coli **(Delves & Gasson, 1994** and **Thomas et al., 1998)**. However, the effect of nisin when combined with various chelators such as citrate, lactate and phosphate is questionable against Gram-negative pathogens particularly *E.coli* **(Catherine & Gregory, 1995** and **Eleiwa- Nesereen, 2003).**

The present study was planned to detect *E.coli* specially shiga toxin producing one in some beef products and their biocontrol through the following topics:-

1. Detection of *E. coli* from the examined meat product samples by conventional method and direct PCR technique.
2. Detection of shiga-toxin genes of isolated *E.coli* by Vero cell cytotoxicity assay and multiplex PCR.
3. Molecular characterization of shiga toxin producing *E.coli* using multiplex PCR.
4. Judgment of the examined samples according to the standards proposed by EOSQ (2005).
5. Assessment the effect of Nisin alone on the growth of *E.coli* O111:H4.
6. Assessment the effect of Nisin combined with sodium acetate on the growth of *E.coli* O111:H4.
7. Assessment the effect of Nisin combined with potassium sorbate on the growth of *E.coli* O111:H4.
8. Assessment the effect of Nisin combined with sodium lactate on the growth of *E.coli* O111:H4

2- Review of literature

2-1- Incidence of *E.coli* especially shiga toxin producing *E.coli* in meat products

Zaki - Eman (1990) isolated 2 strains of *E. coli* from 25 samples of beef burger. Only one strain of Enteropathogenic *E. coli* could be detected. The author also reported that the incidence of *E. coli* in 25 minced meat samples was 44%. The serological typing of *E. coli* revealed that 5 isolates had the classic Enteropathogenic *E. coli* $O_{124:}$ K_{72} (2 strain), O_{44} :K_{74} (2 strain) and O_{127} : K_{63} (one strain).

Gouda (1991) found that the incidence of *E. coli* in the examined minced meat samples was 33%.

Ahmed (1992) detected *E. coli* from 7% out of 40 samples of minced meat. The identified *E. coli* serovars were O_{111}: K_{58} (B_4), O_{86} : K_{61} (B_7), O_{126} : K_7 (B_{l6}) and O_{126} : K_{60} (B_6).

Ghoniem-Amal (1992) isolated *E. coli* from 18% of the examined luncheon samples. The serological identification of the isolated *E.coli* were O_{26} : K_{60} (B_6), O_{78} : K_{80}, O_{111}:K_{58}(B_4) and O_{128} : K_{67} (B_{12}).

Abd EI-Aal (1993) isolated *E. coli* in an incidence of 42.86% from the examined 25 samples of luncheon collected from Assiut and Sohag markets.

Edris (1993) examined 70 random samples of ready to eat meat products including luncheon for detection of *E. coli.* The author identified two different EPEC serovars from luncheon samples which were O_{86} : K_{61} (B_7) and O_{124} : K_{72} (B_{17}).

Nashed-Heba *et al* (1993) examined 125 random samples of frozen meat, minced meat, beef burger, sausage and luncheon for presence of *E. coli*. The incidence of *E. coli* in frozen meat, minced meat, beef burger, sausage and luncheon was 32%, 60%, 24%, 52% and 48%, respectively. The serologically identified *E. coli* were O_{26}:K_{60} (B_6), O_{111}: K_{58} (B_4), O_{55} : K_{59} (B_5), O_{126}: K_7, (B_{16}), O_{86} : K_{61} (B_7) and O_{25} : K_{70} (B_{15}). However, the serovar O_{86} : $K_{6]}$ (B_7) was recovered from minced meat and sausage, whereas, the serovar O_{125} : K_{70} was detected in the examined beef burger samples and the serovar O_{111} : K_{59} was recovered from the examined luncheon samples.

Mousa et al. (1993) isolated *E. coli* from 52%,33% and 45% of the examined luncheon, minced meat and sausage samples, respectively.

Cerqueira et al. (1994) isolated 1066 *E. coli* strains from 105 raw bovine meat samples including minced beef, hamburger and meat balls purchased at supermarkets in Rio de Janeiro, Brazil.

El-Feky (1994) isolated enteropathogenic *E. coli* in a percentage of 9.67% from fresh sausage samples marketed in Sharkia governorate.

Fathi *et al.* (1994) collected 47 random meat product samples from different retail stores and supermarkets in Assiut (19 burger, 16 sausage and 12 luncheon samples). The incidence of *E. coli* isolated from examined burger, sausage and luncheon samples were 9 (47.37%), 4 (25%) and 5 (41.67%), respectively. Whereas the incidence of EPEC strains in the examined samples were 7 (77.78%), 3 (75%) and 4 (80%), respectively.

Aiedia - Hoda (1995) examined 20 samples of traditional Egyptian luncheon and reported that Enteropathogenic *E. coli* was detected in 15% of the examined samples.

Abd EI-Aziz et al. (1996) examined 100 samples of minced meat, beef burger, sausage, pasterma and luncheon (20 of each) collected from different shops in Cairo and Giza. They detected Enteropathogenic *E. coli* (EPEC) in beef burger and sausage with 5% and 10%, respectively. While , Enteropathogenic *E. coli* was not be detected in the examined luncheon samples.

Mohamed (1997) examined bacteriologically 60 random samples of beef burger and sausage (30 of each) and isolated *E. coli* from 6.6% and 10% of the examined beef burger and sausage samples , respectively.

Hassan (1999) collected 80 random samples of minced meat, beef burger, sausage and luncheon (20 of each) from different localities in Kalyobia governorate for detection of pathogenic bacteria. He isolated 2 strains of *E. coli* which are recorded as O_{124}: K_{72} ($B17_{)}$ and $O_{128:}$ K_{67} (B_{12}) from the examined minced meat samples. Moreover, O_{78}: K_{80} was isolated from beef burger and sausage

samples (5% of each) while O_{128}: K_{67} (B_{12}) strain was detected in 5% of luncheon samples.

Uyttendaele et al. (1999) developed a PCR assay targeting the 3 ends of the *eae*-gene of *E. coli* O157:H7. It was shown to be specific for the *E. coli* O157:H7 *eae*-gene. Sensitivity of the PCR assay was 10 cfu per PCR reaction. Subsequently, a study was conducted to examine the effect of the food matrix and the sample preparation method on PCR detection of non-viable cells using heat-killed *E. coli* O157:H7 in ground beef as a model system. Inoculated ground beef samples were subjected to either selective enrichment or immediately prepared for PCR analysis.

Call et al. (2001) used PCR and DNA microarray as a rapid technique for detecting and genotyping of *E.coli O157:H7*. It was considered that glass-based microarrays are relatively simple to construct and provide a rapid and sensitive means to detect multiplexed PCR products.

Fantelli and Stephan (2001) examined 400 minced meat samples collected from 240 small butcheries in Switzerland were collected and analysed for the presence of Shiga toxin-producing *E. coli* (STEC). The samples comprised 211 samples of minced beef and 189 samples of minced pork. Shiga toxin-producing *E. coli* was isolated from 7/400 (1.75%) samples. In particular, 5/211 (2.3%) minced beef samples and 2/189 (1%). minced pork samples were contaminated. Serotyping of the examined 7 strains yielded 5 different serovars, but none of the strains belonged to O157:H7. Two STEC strains harboured *stx*1 and *stx*2 and 5 strains harboured *stx*2c genes. Furthermore, four strains

harboured one or more additional virulence factors. However, none of the strains was positive for *eae*.

Fathi and Thabet (2001) examined 60 random samples of beef burger and sausage (30 of each) for recovery of *E.coli.* The obtained results revealed that 2 strains of *E. coli* were isolated from beef burger samples, while the incidence in the examined sausage samples, was 5 (16.67%).

Ouf – Jehan (2001) examined 60 random samples of hamburger, sausage and luncheon (20 samples of each) collected from different localities in Giza and Cairo governorates. The obtained results revealed that the incidence of *E. coli* in hamburger, sausage and luncheon was 30% and 25% respectively, while in the examined luncheon samples, *E. coli* failed to be detected.

Saleh (2001) examined 100 random samples of meat products (minced meat, burger, kofta and sausage) collected from different local markets in Giza and Cairo governorates for the presence of enterohaemorrhagic *E. coli* (EHEC). EHEC was only recovered from 16% of the examined samples and 5 strains (31.3%) of them were identified serologically as *E. coli* O_{157}: H_7.

Sayed et al. (2001) examined 50 random samples of luncheon collected from supermarkets and restaurants at Assiut city for the presence of *E. coli* especially *E. coli* O_{157} *:* H_7.They found that *E. coli* was detected in a percentage of 30%, while *E. coli* O_{157} *:* H_7 failed to be detected in the examined luncheon samples.

Eleiwa - Nesreen (2003) collected 75 random samples of beef burger, sausage and luncheon (25 of each) from different supermarkets in El-Gharbia

governorate for detection of food poisoning bacteria. The obtained results revealed that *E. coli* failed to be detected in the examined beef burger samples; while it could be isolated from 12% and 4% of the examined sausage and luncheon samples , respectively.

Zaki-Eman (2003) examined 40 random samples of beef burger and sausage (20 of each) collected from different localities in Giza and Cairo governorates. The obtained results revealed that, the incidence of *E. coli,* in the examined beef burger and sausage samples was 35% and 40%, respectively.

Abou- Hussien- Reham (2004) examined 100 random samples of luncheon, sausage, beef burger and frankfurter (25 samples of each) collected from different supermarkets in Kalyobia governorate for detection of pathogenic bacteria. The obtained results revealed that, the incidence of *E. coli,* in the examined luncheon, sausage and beef burger samples was 40%, 12% and 64%, respectively. The serological identification of the isolated *E.coli* in the examined luncheon samples were belonged toO_{86} : K_{61} (B_7) 3 (12%) , O_{55} : K_{59} (B_5) 1(4%), O_{127} : K63 (B_8) 2(8%), O_{111} : K_{58} (B_9) 1(4%), O_{128} : K_{67} (B_{12}) 1(4%) and 2(8%) as well as untypable strains. Moreover, the serological identification of *E.coli* in the examined beef burger samples revealed that 2 strains of *E.coli* (8%) were O_{126} : K_{71} (B_{16}) and 1(4%) was O_{124} : K_{72} (B_{17}). However, the serological identification of *E.coli* in the examined sausage samples showed that only one strain of *E.coli* (4 %) was O_{55}: K_{59} (B_5), 4(16%) o_{128}: K_{67} (B_{12}), 2(8%) O_{124} : K_{72} (B_{17}), 1(4%) O_{125} : K_{70} (B_{15}), 3(12%) O_{119} : K_{69} (B_{19}), 1(4%) O_{26} : K_{60} (B_6) , 1(4%)O_{78} : K_{80} (B_-) and 3(12%) were untypable strains.

Stampi-Serena (2004) investigated the incidence of *E. coli* and *E. coli* O157 in meat samples collected from randomly chosen stores throughout the city of Bologna and suburban areas. For *E. coli* detection, Tryptone soya broth (TSB) supplemented with novobiocin. Vero cell assay and polymerase chain reaction (PCR) were used to assess toxin production and the presence of virulence genes. *E. coli* was detected in 45 (30%) of the examined 149 samples, mainly in the hamburger samples mixed with vegetables and in the loose minced beef. *E. coli* O157 was found in one sample of hamburger and two samples of hamburger mixed with vegetables (2%). All the strains of *E. coli* O157 and most cases of *E. coli* were found in meat from small retailers. The three strains of *E. coli* O157 were positive for verocytotoxin production. PCR analysis revealed genes coding for vt2 and one strain possessed the gene for eae A.

Li et al. (2005) applied a multiplex PCR for simultaneous detection of the pathogenic bacteria in certain raw and ready-to-eat meat matrices. The tested samples had aerobic plate counts ranging from non-detectable, in chicken nuggets and salami, to 8.36 log10 CFU/g in ground pork. The pH of homogenates spanned from 6.86, in ground beef, to 7.17 in salami. Following 24-h enrichment, the multiplex PCR assay could concurrently detect the pathogens at 0.2 log10 CFU/g in ground beef, roast beef, beef frankfurters, chicken nuggets, salami and turkey ham, and 1.2 log10 CFU/g in ground pork. They concluded that multiplex PCR offers an efficient microbiological tool for presumptive detection of *E. coli* in meat.

Abou- Hussien- Reham (2007) examined 40 samples from each of beef burger and beef sausage by using PCR technique and found that pathogenic

E.coli were detected in 20% and 17.5%, respectively. She also revealed that the serological identification of *E.coli* isolated from the examined beef burger samples were 1(2.5%) O_{26}:K_{60} (B_6) ETEC, 2(5%) O_{119}:K_{69} (B_{19}) EPEC, 1(2.5%) O_{124}:K_{72} (B_{17}) EPEC and 4(10%) O_{128}:K_{67} (B_{12}) ETEC. While, the serological identification of *E.coli* isolated from the examined beef sausage samples were 2(5%) O_{26}:K_{60} (B_6) ETEC, 2(5%) O_{86}: K_{61} (B7) EPEC and 3(7.5%) O_{125}:K_{70} (B_{15}) EPEC.

Hassan (2007) examined 160 random samples of luncheon, beef burger, minced meat and fresh oriental sausage (40 of each). The highest incidence of *E. coli* was demonstrated in fresh sausage and minced meat by 13 samples (32.5%) while in beef burger, the incidence was 6 samples (15%) and in luncheon 3 samples (7.5%) . The serovars isolated from beef burger included O119: K69 (B19) and O128: K67 (B12) and their characters were EPEC and ETEC, respectivey for the previously isolated *E. coli*. The *E. coli* serovars isolated from fresh sausage included O78: K80 of ETEC characters, untypable serovars, O126 : K71 (B16) of EPEC, O55 : K59 (B16) of EPEC; O111 : K58 (B59) of EHEC (B59), untypable serovars and finaly the serovars O55 : K59 (B5) of EPEC characters.

Roldan et al. (2007) stated that shiga toxin -producing *E. coli* (STEC) is an emergent pathogen associated with food borne diseases especially food stuffs of animal origin. A total of 250 beef samples (ground beef and hamburger) obtained from Santa Fe and Santo tome cities (Spain) were analyzed by selective enrichment and immunomagnetic separation. *E. coli* 0157:H7 were isolated from 1.2% of the beef samples. These finding confirm the role of food of animal origin in the epidemiology of O157:H7 associated diseases.

Bhong et al. (2008) tested 39 *E. coli* isolates belonging to 35 serovars isolated from market meat products, to find out the prevalence of virulence determinants, Verotoxin 1 (VT1), Verotoxin 2 (VT2), Intimin (eae) genes and enterohemolysin production. Real Time PCR based detection was carried out for virulence genes. Prevalence of VT1 gene in these isolates was much higher (38.70%) . On the other hand, that of VT2 gene was nil (0%) while eae was very low (3.22%). Enterohemolysin production was found in 31.18% of the isolates when tested on washed sheep blood agar supplemented with CaCl2. All enterohemolysin producing isolates were also positive for the VT1 gene.

Ismail (2008) examined 50 samples of beef burger for presence of *E. coli* and coliforms. He found that *E. coli* could not be detected in all the examined samples.

Geoff et al. (2008) examined minced meat used for processing of burger for the prevalence of *E.coli*. The positive samples were 70% from total examined samples, with mean count value of 2.1 x 10 cfu/g on positive samples.

Abou- Hussien- Reham (2008) recorded that *E.coli* was isolated from 57 (45.6 %) of the total examined 125 raw beef burger samples.

Azoz- Afaf (2009) examined 30 samples from each of minced beef, sausage, beef burger and found that *E.coli* was detected in percentage ranged from 10 – 13.3%. .*E.coli O157:H7* failed detection in all examined samples of minced beef and beef burger.

Abongo and Momba (2009) investigated the prevalence of *E. coli* in selected meat and meat products (45 samples each of biltong, cold meat, minced

meat, and polony). Strains of *E. coli* were isolated by enrichment culture and confirmed by polymerase chain reaction (PCR). Also , they investigated the antibiogram profiles of the *E. coli* isolates. Five (2.8%) out of examined 180 meat and meat products were positive for *E. coli* that carried the fli CH7, rfbEO157, and eaeA genes.

Lee et al. (2009) investigated the prevalence of *E. coli* contamination in fresh beef, poultry, and pork, and to determine whether any isolated *E. coli* possessed genes associated with pathogenicity. Three thousand meat samples were collected from 2004 to 2006 and were tested for the presence of *E. coli*. Two hundred and seventy-three *E. coli* isolates were obtained from beef, poultry, and pork, resulting in an overall isolation rate of 9.1% , of these isolates, 201 were obtained from 1350 pork samples (14.9%), followed by 41 of 900 poultry samples (4.6%) and 31 of 750 beef samples (4.1%). A total of 39 pathogenic *E. coli* isolates from the three meat types were categorized into three virulence groups, namely enterotoxigenic *E. coli* (43.6%), enterohemorrhagic *E. coli* (EHEC) (35.9%; 22.6% of beef, 7.3% of poultry, and 2.0% of pork), and enteropathogenic *E. coli* (20.5%). Fourteen strains were identified as belonging to the EHEC, which included O18, O136, O119, O86, O8, O111, O15, O128, and O6. It was demonstrated that pathogenic *E. coli* are found in meat in Korea, and could act as a transmission vehicle for human infection as suggested by the occurrence and classification of pathogenic *E. coli* in retail meats. Furthermore, the data from this study was used in the risk assessment of foodborne illnesses linked to meat consumption.

Sarimehmetoglu et. al. (2009) analyzed 251 fresh ground beef samples sold in Ankara, to evaluate the prevalence of *E. coli* O157:H7 *by* immunomagnetic

separation (IMS) based cultivation technique. Virulence factors of the isolates were determined by multiplex PCR. Nineteen (7.6%) of 251 ground beef samples were found as contaminated with *E. coli O157*. According to the multiplex PCR one of the isolate has all stx1, stx2, eaeA, hly and fliCh7 genes and the other has stx1, eaeA, hly and fliCh7 genes.

Takahashi et al. (2009) developed rapid enumeration of *E.coli* strains by quantitative real-time PCR targeting the uidA gene this was confirmed for minced beef, tuna and raw oyster. Higher sensitivity (1 CFU/g of *E. coli* in all three food samples) was obtained by incubating for 7 h in Tryptic Soy broth (TSB). Colony-directed *E. coli* specific TaqMan PCR assay could effectively distinguish colonies grown on various selective media within 1.5-h. It was concluded that inspection of *E. coli* in food testing laboratories is important, and this rapid *E. coli* detection strategy will contribute to quality control in food industries.

Gordillo et al. (2011) developed a multiplex polymerase chain reaction (PCR) procedure based on fliCh7 and rfbE genes for the detection of *E. coli* in raw pork meat and ready-to-eat (RTE) meat products. Two different DNA extraction procedures were evaluated for application on meat products. MasterPure™ DNA Purification kit in combination with immunomagnetic separation was found to be the best method in a meat system. The optimized PCR included an enrichment step in brilliant green bile 2% broth at 37 °C. This method was applied to artificially inoculated meat and RTE meat products with different concentrations of *E. coli*. The results indicated that the PCR assay could sensitively and specifically detect *E. coli* in raw pork meat and RTE meat products in approximately 10 h,

including a 6 h enrichment step. Thus, they reported that this method could be proposed for screening *E. coli* O157:H7 in raw pork and RTE meat products.

Mohammed (2012) determined the serological characteristics and virulence-associated genes of 32 *E. coli* strains isolated from different meat products. Serotyping of somatic (O) and flagellar (H) antigens reveled that 3 strains were typed into 2 serogroups; O121:H19 (2 strains) and O148:H8 (1 strain), while the other 29 strains were not agglutinated with any serum. For molecular characterization, multiplex PCR has been performed by combining seven primer pairs specific for enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli* (EIEC). The targets selected for each group were the genes encoding heat-labile (LT) and heat-stable (ST) toxins for ETEC isolates, eae, stx1 and stx2 for EHEC isolates, eae and bfpA for EPEC isolates, and ipaH for EIEC isolates. Such facilitates were simultaneous identification of the four different categories of diarrheagenic *E. coli* in a single reaction. Amongst the 32 tested *E. coli* strains, eleven (37.5%) were potentially diarrheagenic. Five of which (15.63%) were ETEC, three (9.38%) were EHEC, two (6.26%) were EPEC, and one strain (3.13%) was EIEC. The results indicated that virulence gene-carrying *E. coli* strains are a normal part of intestinal bacterial populations that may be present among high numbers of *E. coli* contaminating meat products which do not necessarily correlate with disease.

Hassan (2012) examined 160 random samples of luncheon, beef burger, minced meat and fresh oriental sausage (40 of each) for isolation *E. coli* by using conventional method and PCR. Conventional method indicated that *E .coli* could be isolated from luncheon, beef burger, minced meat, and fresh sausage in a percentage of 22.5%, 32.5%, 47.5% and 60% respectively. While, on using PCR

was detected *E .coli* was detected in percentage of 20%, 32.5%, 42.5% and 57.5% respectively. The serological identification of *E.coli* isolated from the examined luncheon samples were O63 (ETEC), O111 (EHEC) , O124 (EIEC) , O126 (EPEC) and O142 (EPEC) . While for beef burger samples, the isolated serovars were O25 (ETEC), O112 (EIEC), O114(EPEC), O119 (EHEC) and O127 (EPEC). But the isolated serovars from minced meat were O26 (EHEC), O78 (ETEC), O86 (EAEC), O128 (EPEC) and O142 (EPEC).The isolated serovars from fresh sausage were O25 (ETEC), O111 (EHEC), O126 (EPEC), O128 (EPEC) and O136 (EIEC).

Mewafy - Abeer (2012) stated that the incidence of *E.coli* O157: H7 isolated from ground beef, beef luncheon, beef sausage and beef burgers were 5 %, 0.0 %, 0.0 % and 10 % , respectively. Also she investigated the presence of shiga-like toxins stx1 and stx2 among the isolates and she reported that that *E.coli* O157:H7 isolates had stx1, stx2 and hyl genes.

EL-Alfy et al (2013) conducted a study to identify and characterize the virulence traits of enterohaemorrhagic *E. coli* from different sources, between September 2008 and October 2009. A total of 284 samples from environmental sources were collected from different locations in Ismailia, Egypt. *E.coli* isolates (n = 283) were identified by conventional microbiology culture and were phenotypically characterized using biochemical and motility tests. Multiplex PCR was applied for the detection of virulence genes (*stx1*, *stx2*, *eaeA* and EHEC *hlyA*) and they recorded in 6% (17/283) examined processed meat products. Shiga toxin genes were identified in two isolates from meat products (serogroups O125 and O158). This study identified STEC O157 from human cases with diarrhea, and demonstrated that meats could be contaminated with more than one STEC serovars and it is a concern due to their potential to cause human infection.

Mohammed et al (2014) determined the prevalence, serovars and virulence genes distribution of non-O157 shiga toxin-producing *E. coli* in meat products collected from butchers shops and supermarkets in Mansoura city, Egypt. They characterized 18 non-O157 STEC strains among the identified 100 *E. coli* isolates recovered from the examined 87 meat product samples. The prevalence of non-O157 STEC strains in fresh beef, ground beef and beef burger samples were 11.1% (3/27), 16.7% (5/30), and 33.3% (10/30), respectively. The eighteen non-O157 STEC isolated strains were serotyped into seven (38.9%) O111:H8, six (33.3%) O26:H11, two (11.1%) O111:He, and one (5.56%) for each of O55:H7, O126:H5 and O128:H2. PCR assays for different virulence genes showed that nine (50%), eleven (61.1%), and nine (50%) strains carry stx1, stx2, and eae genes, respectively. The distribution of shiga toxin genes among the isolated strains indicated that seven (38.9%) strains harbored stx1 only, nine (50%) strains harbored stx2 only, and two (11.1%) strains harbored both stx1 and stx2. The eae gene was present in association with five (27.8%), three (16.7%), and one (5.6%) strains that harbored stx1 only, stx2 only, and both stx1 and stx2, respectively.

2-2-PCR technique for detection and confirmation of *E.coli* in meat products.

Bej et al. (1991) recorded that the PCR is a method of DNA amplification where a target DNA sequence is exponentially multiplied to enable its detection. The role of PCR from the perspective of food safety testing is that it is significantly closer to real time than standard culture methods. DNA replication in a test tube requires less than 2 minutes compared to the 30-60 minutes, replication by bacterium. The target sequence is selected using primers, which are

single-stranded DNA fragments that anneal to the target sequence, nucleotides, and DNA polymerase. They are mixed with the sample and put through a series of heating and cooling cycles. During heating, DNA is separated into single strands, and during cooling, the primers bind to the target sequences. In the next heating the DNA polymerase uses the nucleotides to extend the primers, creating a copy of the DNA fragment.

Olsvik et al. (1991) stated that PCR technologies can be useful tools in making this type of diagnosis efficient and provide information of epidemiological importance, especially with respect to routes for spreading pathogenic *E.coli* strains with food as a vector.

Shiral et al. (1991) stated that PCR is highly sensitive and specific technique used for the rapid identification of pathogens, especially that in vitro cultivation is difficult or not possible. It also detected that activities rather than the simple presence of microorganisms. PCR can be applied to fixed tissues (frozen or formalin fixed), reducing the potential dangers involved in transport and handling of specimens with live virulent pathogen, It is one of the most promising techniques available for rapid detection of food microorganisms that substituting the biochemical or serological characterization of the pathogen by the DNA analysis.

Wernar et al. (1991) used PCR as a tool for detection of ETEC in minced meat with 2 synthetic 20-meroligonucleotides, a 195-bp fragment from the *E.coli* Liable Toxin (LT) could be amplified specifically. When 6 CFU was added to the reaction mixture as a template, the PCR yielded sufficient amplified product for visualization as an agarose gel. Prior to PCR amplification, the minced meat

samples were subjected to enrichment culturing for *E.coli*. Out of this culture, 10 uL was used in the PCR assay. All 25g samples that were examined in such assay were negative for *E.coli* LT. However, when 3 CFU of *E.coli* was added to 25g samples of minced meat prior to enrichment culturing, the PCR assays yielded positive results.

Olsvik and Strockbine (1993); Fratamico et al. (2000); Ibekwe et al. (2002) and Ibekwe and Grieve (2003) found that molecular approaches for bacterial detection avoid the need for culture and can be designed to be specific. Primers specific for stx1 and stx2, as well as *E. coli* O157:H7-specific targets, have been used in PCR and real-time PCR.

Candrian (1995) stated that the PCR is one of the most promising techniques available for rapid detection of food and environmental microorganisms. PCR is a technique for in vitro amplification of DNA sequences that are preferably unique to the organism of interest. Beginning with DNA of any origin (bacteria, viral, plant and animal) PCR can increase the amount of the targeted DNA sequences 10^6 to 10^9 times. The procedure, which based on an enzymatic process, has an application range down to 1 ppm of DNA in the initial sample, thus being of great use in the detection of very small DNA quantities. It is concluded that PCR technology has significantly reduced the detection time for pathogen identification in food to approximately 2 days, but still the only disadvantage of PCR is its high expensive.

Flanders et al. (1995) stated that cultural enrichment of food samples prior to DNA extraction made PCR technique holds several advantages over direct detection methods. Although enrichment steps increase analysis time, significant

increases in sensitivity and specificity of the PCR assay can be accomplished using this step. PCR inhibitors are diluted out in the enrichment step, along with non-target and dead microorganisms. Moreover, selective enrichment can increase the concentration of target microorganisms and eliminate competition between background flora present in the sample. The disadvantage of using enrichment step before DNA analysis is the idea that only culturable bacteria will be detected. Heat injured and stressed bacteria may be viable, but not able to withstand the selective conditions of some enrichment media.

China et al. (1996) stated that the conventional culture methods have traditionally been considered the "gold standards" for the isolation and identification of foodborne pathogens. However, culture methods are labor-intensive and time-consuming. PCR assay for the detection of *E.coli* and Salmonella in a variety of food and food-animal matrices was applied. The real-time PCR assay based on the sequence of the *E. coli* and Salmonella gene ensure highly sensitive and specific results.

Gilgen et al. (1998) stated that pathogenic strains of *E. coli* have been recognized as a cause of human disease, and rapid and sensitive detection tests are urgently needed to ensure the safety of food, especially ground beef. The author applied two tested polymerase chain reaction (PCR) assays to detect the genes encoding VT1 and VT2 irrespective of the bacterial serovars. The authors were able to uncover the presence of about 110 CPU of verotoxinogenic *E. coli* (VTEC) in 10 g of ground beef. When six-hour enrichment was included, they found that the detection limit to be in the range of 1 to 10 bacterial cells per 10 g

of ground beef. The results suggested that the described PCR method can serve as a valuable tool for the surveillance of VTEC contamination of food.

Karch and Bielaszewska (2001) stated that rapid methods to detect *E. coli* O157:H7 are important to identify the source of outbreaks and to assure public safety. Both molecular and culture-based methods have been used for the detection of *E. coli* O157:H7. Culture-based methods developed for clinical samples have been applied to environmental samples. These methods rely on enrichment cultures followed by confirmation based on metabolic and antigenic properties. A disadvantage of this approach is the lack of complete correlation of these antigenic and metabolic properties with Stx production.

Payton et al. (2002) indicated that more aggressive sampling plans and genetic screening technologies such as PCR may be used for better detection of low levels of *E.coli* O_{157}:H_7 in ground beef products.

Blanco et al. (2004) studied a total of 153 Shiga-toxin-producing *E. coli* (STEC) isolates from beef products (burgers and ground beef) and others and reported that PCR thchnique showed that 22 (14%) isolates. carried stxl genes.

Mi-Yeong Ji et al. (2004) investigated the prevalence of *E. coli* O157 in meat samples of cattle, in Korea from April, 2000 to July, 2002. Eighty-six (3.03%) of 2843 samples were positive for *E.coli* O157. Out of 86 *E.coli* O157 isolates, 73 were serovar O157:H7 and 13 were serovar O157. Polymerase chain reaction (PCR) analysis of *E.coli* O157 virulence markers revealed that all O157:H7 isolates were positive for EhlyA, eaeA and rfbO157, and 77 isolates were positive for stxl and/or stx2. Cytotoxicity analysis revealed that many of the *E.coli* O157 isolates

showed high cytotoxicity on Vero cells. The obtained data suggested that the majority of Korean *E. coli* O157 isolates from food animals can cause serious diseases in human being.

Tasai et al. (2006) mentioned that enterohemorrhagic *E. coli* O157:H7 is an infectious pathogen and outbreaks have been reported all over the world, in various countries in Europe and South Africa. The pretreatment protocol, which included immunomagnetic separation (IMS) to concentrate and purify the *E. coli* O157, was developed and the sensitivity of the assay was improved to 10 CFU/ml in pure culture and food samples. The TaqMan PCR assay is a rapid test for the detection of *E. coli* O157 in food matrix. Where it shortens the process time and increases the specificity of the pathogens detected. Where it critical for improving the safety and sanitation of our food supply.

Jeong Soon et al. (2007) stated that rapid and sensitive detection techniques for foodborne pathogens are important to the food industry. However, traditional detection methods rely on bacterial culture in combination with biochemical tests was a process that typically takes 4 to 7 days to complete. Such study was conducted to address the issue of time lag inherent in traditional methods by developing a novel PCR assay for each of five foodborne pathogenic bacteria. Such new system consists of a simultaneous screening method using multiplex PCR in a single reaction tube for the rapid and sensitive detection of each of the five bacteria. Specific primers for multiplex PCR amplification were used. Genes were designed to allow simultaneous detection of *E. coli* O157:H7 and Salmonella, respectively. In the multiplex PCR with mixed DNA samples, specific bands for corresponding genes were simultaneously detected from a

single reaction. The detection of all foodborne pathogenic bacteria could be completed in less than 24 hours with this novel PCR method.

Guion et al. (2008) stated that diarrhogenic *E.coli* strains are important causes of diarrhea in children from the developing world and are now being recognized as emerging enteropathogens in the developed world .Current methods of detection are too expensive and labor-intensive for routine detection of these organisms to be practical. The authors developed a real-time fluorescence-based multiplex PCR for the detection of all six of the currently recognized classes of diarrheagenic *E.coli.* Eighty-nine of ninety diarrheagenic *E. coli* and nonpathogenic *E. coli* strains were correctly identified using this approach (specificity, 1.00; sensitivity, 0.99). The total time between preparation of DNA from *E. coli* colonies on agar plates and completion of PCR and melting-curve analysis was less than 90 min. The cost of materials was low. Melting-point analysis of real-time multiplex PCR is a rapid, sensitive, specific and inexpensive method for detection of diarrhogenic *E. coli*.

Azoz- Afaf (2009) used PCR as a method for identification of *E. coli* and Salmonellae and found that PCR was effective, less labor, more sensitive and reduce effort and time. PCR method revealed that the isolated *E. coli O157:H7* had the ability to produce Shiga like toxin type one (Slt1).

Gordillo et al., (2011) stated that PCR is considered rapid technique for detection of *E.coli* as it can detect it within 10 hours, including a 6 hours enrichment step.

Hassan (2012) used rapid PCR as a method for identification of *E. coli* in meat products and found that PCR was effective, less labor, more sensitive and reduce effort and time.

Son et al.(2014) used multiplex PCR for detection of non-O157 Shiga toxin-producing *E. coli* (STEC) belonged to serovars O26, O103, O111, O145,O157, O91, O113, O128, O45, and O121 as well as detection of five Shiga toxin-producing *E.coli* genes with multiplex PCR.

2.3.*Shiga* toxins and virulence genes of *Escherichia coli*.

Konowalchuk *et al*. (1977) recorded that *Escherichia coli* was the archetypal serotype for a series of *E.coli* strains capable of producing at least two distinct extracellular cytolytic Shiga-like toxins (Stx1 and Stx2).

Siegler (1995) stated that after of food contaminated with *E.coli* O157 entered the intestines where it attached to the intestinal mucosa and produces Shiga-like toxins (Stx1 and Stx2). These toxins caused the wall of the intestine to become porous, allowing further toxin to enter the bloodstream and induced the clinical manifestation known as HUS.

Su and Brandt (1995) reported that *E.coli* O157:H7 was serologically determined by two antigens. The somatic antigen O157 reflected the nature of the phospholipid-polysaccharide complex and the order in which it occured in the repeating units of the polysaccharide chain. The flagellar antigen H7 was determined by the nature of the repeating flagellar subunits, flagellin. *Escherichia*

coli O157:H7 caused a wide spectrum of clinical symptoms including hemorrhagic colitis (bloody diarrhea), hemolytic-uremic syndrome (HUS), non-bloody diarrhea and Thrombotic Thrombocytopaenic Purpura (TTP).

Paton and Paton (1998) recorded that synthesis and secretion of the *Shiga* toxins was the most potent virulence feature of *E.coli* O157:H7. The toxin name was derived from the prototype Shiga toxin from *Shigella dysenteriae*. Not only *E.coli* and *S. dysenteriae* could produce Shiga toxins; but also *Citrobacter freundii, Aeromonas hydrophila, Aeromonas caviae* and *Enterobacter cloacae* had been reported to be able to express these toxins as well.

Schmidt *et al.* (2000) stated that the two main types of toxins, Stx1 and Stx2, were produced by *E.coli* O157:H7. Furthermore, several Stx2 variants had been found, including Stx2c, Stx2d, Stx2e and Stx2f, which ranged from 63-99 % identity in the A subunit and 75-95 % identity in the B subunit of the Stx2 toxin .

Gruenheid *et al.* (2001) recorded that the Locus of Enterocyte Effacement also contains the *tir* gene, which encoded the translocated intimin receptor. After the translocation of *tir* protein from the *Escherichia coli* O157:H7 cell to the host cell cytoplasm via the EspA tube, it localizes to the plasma membrane of the host cell where it served as the receptor for attachment of the bacterium. In addition to serving as a receptor for intimin, the 78-kDa plasma membrane-bound *tir* coordinated remodeling of the host cell cytoskeleton by binding to the host cell protein Nck.

Hengge-Aronis (2002) recorded that *E.coli* in general had multiple genetic systems that responded to physical and chemical challenges and that

confered resistance to low and lethal pH. The σs (or RpoS) subunit of RNA polymerase was a master regulator of the general stress response in *E.coli*. While nearly absent in rapidly growing cells, σs was strongly induced during entry into stationary phase and/or many other stress conditions, and was essential for the expression of multiple stress responses .

Bouzari et al. (2007) studied pathogenecity of *E.coli* and can involve a large number of virulence factors, toxins being the most obvious .They assessed the distribution of genes encoding toxins among *E.coli* isolates from diarrhoeal cases using DNA probes. From 200 isolates, 92 (46.0%) carried genes encoding for toxins, 43.5% of these being multitoxigenic. Enteroaggregative heat -stable enterotoxin producing strains were detected in 40 (43.5%) of isolates. Verotoxin in 38 (41.3%),cytolethal distending toxin in 24 (26.1%),heat-stable enterotoxin in 12 (13.0%)and heat–labile enterotoxin in 10 (10.9%),furthermore,40strains (70.0%) are resistant. They concluded that the toxigenicity and antibiotic resistance are the main contributing factors leading to the virulence potential of these *E.coli* isolates.

Kansas Disease Investigation Guidelines (2011) recorded that more than 100 serovars of *E. coli* produce Shiga-like toxins. The most commonly identified Shiga toxin-producing *E.coli* (STEC) in North America is *E.coli* O157:H7 which was first identified in 1982. In the U.S., it is estimated that 70,000 infections per year were caused by *Escherichia coli* O157. Persons of all ages were susceptible and very young children and the elderly were more likely to develop severe illness and hemolytic uremic syndrome (HUS).

Ursula et al. (2012) reported that Shiga toxin (stx)–producing *Escherichia coli* (STEC) was among the most common causes of foodborne diseases. The role of non-O157 STEC strains (e.g., O26:H11/H^-, O91:H21/H^-, O103:H2, O111:H^-, O113:H21, O121:H19, O128:H2/H^-, and O145:H28/H^-) as causes of HUS, bloody diarrhea, and other gastrointestinal illnesses was being increasingly recognized .

2.4. Public health and economic importance of isolated *E.coli* especially shiga toxin producing *E.coli*:

Schloger et al. (1990) stated that non-toxigenic and colonizing strains of *E.coli* distincted from the recognized pathogenic types which like enteroadherent-aggregative strains were involved in persistent diarrhea as a major cause of illness. Further types of diarrhoeagenic *E.coli* may be recognized as an important human pathogen.

Collins et al. (1991) found that *E.coli* strains were associated with human and animal infections and proved to be the most common cause of urinary tract infections ,suppurative lesions, neonatal septicemia and meningitis in human .

***FAO / WHO (1991*)** stated that *E.coli* constitute part of the normal flora of the intestinal tract of human being and most of the warm blooded animals , however there are pathogenic strains that cause distinct syndromes of diarrheal diseases and that have been associated with foodborne illness .

Griffin and Tauxe (1991) reported that the enterohaemorrhagic *E.coli* (EHEC) was responsible for abdominal cramps, diarrhea and in limited case of haemolytic uremic syndrome (HUS) which occur most often in children and /or 5 years . The illness was fatal in 3 to 5% of cases They attributed acute kidney failure occurred in children to this illness .

Varnam and Evans (1991) mentioned that enteropathogenic strains of *E.coli* are responsible for many cases of infantile diarrhea . In such cases , the severity of symptoms varied from severe to mild , which may pass undetectable to severe and possibly life threating .

Gordillo et al. (1992) pointed out that enteroinvasive *E.coli* (EIEC), is an important cause of endemic diarrhea in South America and Eastern Europe .It was responsible for 5% of cases of diarrhea in a study of U.S. travelers to Mexico. It has been implicated in occasional food-borne outbreaks of enterocolitis in adults in industrialized countries, with three outbreaks have been reported in the United States. The largest of these was an epidemic of gastroenteritis affecting at least 226 persons in 96 outbreaks throughout the country.

Siegler (1995) stated that hemorrhagic colitis is the most common symptom associated with *E.coli* O157:H7, typically appearing 1 to 5 days after ingestion with most patients recovering within 10 days. In some cases, infection may result in life-threatening complications such as HUS and TTP. In addition, kidney damage occurred from which about 50% of HUS patients suffer acute kidney failure and require dialysis.

Mossel et al. (1995) reported that *E.coli* is one of the cause of febrile types of gastroenteritis transmitted by foods. They tabulated O111 and O127 as

member of group containing major virulence factors. Some considered O111 serovar as verotoxin producers and some considered it as enterohaemorrhagic types, while O127 was tabulated as devoid of toxin production, but gives rise to attachment lesions in intestinal mucosa, destruction in intestinal epithelium and microvilli causing profused diarrhea.

Armstrong et al. (1996) stated that *E.coli* O157:H7 was currently the most common EHEC strain in many regions of the world.

Bettelheim (1996) said that *E.coli* can cause a number of intestinal illness in human including bloody diarrhea and haemolytic uraemic syndrome . These organisms produce a number of virulence factors particularly the shiga – like toxin (verotoxins) . The intestines of animals may be the reservoir of these organisms for human infection and cattle particularly they have been shown to harbour them .

Paton et al. (1996) reviewed that enterohemorrhagic *Escherichia coli* strains, especially strain O157, had a low infectious dose of 1 to 100 CFU/g.

Itoh et al. (1997) reported that a massive outbreak of gastrointestinal illness occurred in Tajimi city ,Japan, in June of 1993 in which 2,697 children in elementary and junior high schools developed severe diarrhea. Twenty-seven strains of enteroaggregative *E.coli* (EAggEC) isolated from stool specimens of 12 of 30 patients were belonged to the same serotype, O untypeable: H10, and showed the same biochemical characteristics and antibiotic susceptibility pattern. The authours suggested that the EAggEC serovar: H10 was associated with this massive outbreak of gastrointestinal illness.

Shere et al. (1998) recorded that the infections of humans with the *Escherichia coli* O157:H7 serotype occurred most frequently as food-borne outbreaks. Food safety had since become a presidential-level priority. Traditionally, industry and regulators had depended on spot-checks of manufacturing conditions and random sampling of final products to ensure safe food.

Mitsuda et al. (1998) pointed out that ETEC serovars most commonly associated with large outbreaks in Japan were O6:NM and O6:H16, which produce both heat stable (ST) and heat-labile (HL) toxins, as well as O27:H7, O27:H20 and O159:H20 which produce only ST. ETEC . Serovars O8, O25, O148 and O167 have also been reported in the literatures.

***Roels et al. (1998*)** recorded that , in September 1994, a foodborne outbreak of enterotoxigenic *E.coli* (ETEC) infection occurred in attendees of a banquet in Milwaukee ,USA. *E.coli* was isolated from stool specimens from 13 patients that were comprehensively tested. Isolates from five patients positive for *E.coli* producing heat-stable toxin, were biochemically identified and serovars as *E. coli* O153:H45 were identified . Diarrhea (100%) and abdominal cramps (83%) were the most prevalent symptoms in 205 cases while vomiting (13%) and fever (19%) were the less common. The median duration of diarrhea and abdominal cramps was 6 days and 5 days, respectively .The authors concluded that in the United States, health care providers rarely consider ETEC as a possible cause of diarrhea in their patients, and few laboratories offer testing to identify ETEC. Hence, outbreaks of ETEC infection may be underdiagnosed and underreported.

Baljer and Wieler (1999) stated that EHEC were recognized as zoonotic pathogens only since 1982. EHEC are capable of causing hemorrhagic colitis and some sequelae of diseases such as the haemolytic uraemic syndrome. EHEC are one of the world wide most important causes of foodborne infections. In human the symptoms of the disease appear as aqueous to bloody diarrhea.

Mead *et al*.(1999) estimated that *E. coli* O157:H7 caused 73,000 illnesses annually in the United States and non-O157 STEC caused 37,000 illnesses and that 91 deaths occured each year in the USA.

Hundson et al. (2000) reported that 10% of human cases of HUS in New Zealand were caused by non-O157 STEC. Consequently, the use of O157 specific isolation or detection methods incurs the risk that outbreaks and food poisoning incidents caused by non- O157 STEC may be undetected. The authors recommended enterohemorrhagic *E.coli* agar (EHEC) as a medium of choice for detection of all STEC serovars.

Paredes et al. (2000) studied the etiology of diarrhea among 332 travelers to five all-inclusive hotels in Negril, Jamaica, U.S.A., between December 6, 1994 and March 10, 1996 and found that ETEC isolations (68%) occurred as part of a clustering of diarrhea cases. The largest outbreak of pathogen-identified diarrhea consisted of 7cases of ETEC producing both heat-stable and heat-labile enterotoxins.

WHO (2000) found that up to 30% of people in developing countries suffer from foodborne illness and children under the age of 5years were died of diarrheal diseases in 1999 ,mostly due to *E.coli* .

Chakraborty et al. (2001) reported that in Ahmedabad, a major city in the state of Gujarat, India, an outbreak of acute secretory diarrhea caused by *Vibrio cholerae* O1 Ogawa El To, *V. cholerae* O139, and multiple serovars of enterotoxigenic *E.coli* (ETEC) occurred in January 2000. ETEC isolates of different serotypes were positive for the elt gene, encoding heat-labile enterotoxin. The authors concluded that this was a unique outbreak, and that it was the first in which *V- cholerae* and ETEC were concomitantly involved.

El-Sheikh and El-Assouli (2001) investigated the viral, bacterial and parasitic pathogens among children of Jeddah, Saudia Arabia during December 1995 to October 1996, suffering from acute diarrhea. One of the etiological causes was *E.coli* (13%) of which 3.8% were Enteropathogenic *E.coli* (EPEC) and 19% enterohemorrhagic *E. coli* (EHEC).

Cortes-Ortiz et al. (2002) examined rectal samples collected from the population of Chalco valley, Mexico, who suffered from diarrhea and vomiting during a natural disaster that took place on May 31, 2000.They could isolate ETEC (62.2%), EIEC (0.84%), EPEC (0.84%), and EHEC non-O157:H7(.08%). Enterotoxigenic *E.coli* was the most likely etiologic agent. The authours stated that sanitary control strategies should be targeted to prevent outbreaks caused by this pathogenic agent.

Ochoa and Cleary (2003) stated that children with hemorrhagic colitis routinely developed a spectrum of coagulation abnormalities and that only a fraction of children develop full blown haemolytic uraemic syndrome. They also

added that the lag between the onset of diarrhea and the onset of HUS represents an opportunity to intervene and prevent renal failure.

American Meat Institute (2007) reported that generic *E.coli* bacteria were an essential, but normally harmless component of the digestive tract of healthy animals and people. *E.coli* O157:H7 was a virulent strain of the family of generic bacteria that was found in cattle, deer and other warm-blooded animals.

Bulte (2008) stated that since the first outbreak caused by shiga toxin-producing *E. coli* (STEC) of serovar O157:H7 in 1982, this agent has emerged as afoodborne pathogen leading to haemorrhagic colitis (HC), hemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). In addition to the protopatopathotype O157:H7, other enterohaemorrhagic *E. coli* (EHEC) serovars were (O26:H11, O1003:H2, O111:H-O145:H-and O157:H7 have caused severe infections in humans. Nearly all of STEC starins contain the *E.coli* attaching and affacing gene (eae) encoding the outer membrane protein intimin, which mediates the attachment to enterocytes leading to irreversible destruction of the microvilli.

Fernandez (2008) reviewed that EHEC were first identified as human pathogens in 1982, when *E.coli* of serovar O157:H7 was associated with two outbreaks of hemorrhagic colitis. *Escherichia coli* O157:H7 and many serovars of *E.coli* had subsequently shown to produce VTs; hence they had named VT producing *Escherichia coli* (VTEC).

Hajian et al. (2009) mentioned that according to the Food Research Institute the Shiga toxin-producing *E.coli* (STEC) could cause devastating illness,

particularly in children, by causing hemolytic uremic syndrome (HUS) leading to kidney failure. Outbreaks of illness caused by STEC had been epidemiologically related to contact with animals and consumption of meat and fresh produce. *Escherichia coli* O157:H7 was the most notorious of the STEC strains caused approximately 73,500 cases in the U.S. each year. Center of Diseased Control (CDC) estimated that non-O157 STEC were responsible for about 37,000 cases of illness annually but relatively fewer cases of HUS compared to O157:H7. Although many strains of non- O157 STEC appeared to be less virulent than *E.coli* O157:H7, a 2008 outbreak of STEC strain O111 in Oklahoma caused illness in at least 314 people, HUS in 17 cases, and one death . Other non-O157 outbreaks in the U.S. had been traced to contaminated lake water, salad greens, and milk. Numerous *E.coli* strains were capable of producing one or both Shiga toxins (Stx1 and Stx2), but not all of them were important human pathogens. STEC strains had been divided into 5 seropathotypes. **A**, included the O157 strains that were common causes of outbreaks and HUS in most countries; **B**, non-O157 strains that caused occasional outbreaks but were fairly common isolates from sporadic cases and HUS (examples: O26:H11, O103:H2, O111: NM(no name), O121:H19, O145: NM); **C**, non-O157 strains associated only with sporadic cases; **D**, strains associated with diarrhea, not more severe symptoms; and **E**, strains not associated with human disease. Stx2 is the more potent toxin, and those strains producing this toxin were generally associated with more acute illness.

Shaheen et al. (2009) studied the epidemiology of diarrheal diseases among Egyptian children in Nile River Delta. Enterotoxigenic *E. coli* was isolated from 320/1540 (20.7%), and contain heat stable and heat labile toxins (75%) of the totally collected positive samples.

Abongo and Momba (2009) stated that *E. coli* is a human pathogen worldwide associated with meat and meat products, dairy products, vegetables and water. It is recognized as a bacterium causing hemorrhagic colitis. Diarrheal diseases linked to *E. coli* infections are characterized by blood, cramping abdominal pain, fever, nausea, and vomiting.

Gordillo et al. (2011) stated that enterohemorrhagic *E.coli* is a serious pathogen included in the verocytotoxigenic group of *E. coli*. This serovars has been found to be responsible for a wide range of illnesses in humans, including hemorrhagic colitis and hemolytic uremic syndrome.

Sima et al. (2011) mentioned that *E.coli* O157: H7 was recognized as an important cause of diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome worldwide. Meat, meat products, dairy products, vegetables and drinking water contaminated with animal feces were probably the major sources of the *E.coli* O157: H7 infection.

WHO, (2012) reported that *Escherichia coli* O157:H7 was the most important EHEC serovar in relation to public health; however, other serovars had frequently been involved in sporadic cases and outbreaks. Preventive measures for *E. coli* O157:H7 infections were similar to those recommended for other foodborne diseases. Basic good food hygiene practice, as described in the WHO Five keys to safer food, could prevent the transmission of pathogens responsible for many foodborne diseases, and also protected against foodborne diseases caused by EHEC.

Son et al. (2014) reported that *E.coli* serogroup O157 is the pathogen most commonly associated with foodborne disease outbreaks, but epidemiological studies suggest that non-O157 Shiga toxin-producing *E.coli* (STEC) is amajor player as well. The ten most clinically relevant STECs belong to serogroups O26, O103, O111, O145,O157, O91, O113, O128, O45, and O121; but emerging strains, such as O104:H4 that was identified with the 2011 German outbreak, could become more prevalent in the future.

2.5. Biocontrol of *E.coli* growth in meat products .

Delves and Gasson (1994) and **Thomas et al(1998)** stated that nisin is used in meat technology as a chemical preservative where it has a powerful inhibitory effect against Gram-positive bacteria, but probably has not the same effect on Gram-negative ones as *E.coli* .

Catherine and Gregory (1995) stated that the effect of nisin when combined with various chelators such as citrate, lactate and phosphate is questionable against Gram-negative pathogens particularly *E.coli.*

Demel et al (1996) and Hassan (1999) mentioned that the addition of nisin alone whatever its concentration was not effective for complete destruction of *E.coli* inoculated into minced beef samples.

Elliason and Tatini (1999) mentioned that nisin enhanced inactivation of *Escherichia coli* O157:H7 when stored at 6.5°C, as the presence of 100 IU nisin induced a significant decrease of 1.5 log cycles in cell numbers after 14 days. They

added that nisin might be a mean for providing an additional barrier to enhance the safety of refrigerated food, as a refrigerated storage could cause sufficient injury to sensitize Gram-negative pathogens to nisin.

Ganzle et al (1999) stated that the resistance of *E.coli* as Gram-negative bacteria against nisin depends on the type of lipopolysaccharid of the cell membrane. Consequently, addition of an agent to change the nature of the outer membrane of *E.coli* is very necessary to render the organism sensitive to nisin**.**

Tipayanate et al (1999) mentioned that the incorporation of nisin with sodium lactate lead to a complete destruction of *E.coli* after 24 and 48 hours of this treatment. It is postulated that sodium lactate destabilize the cell membrane of *E.coli* and other Gram-negative bacteria by chelating of Mg++ and /Ca++ affecting its permeability to be sensitive to the action of nisin **.**

Eleiwa- Nesreen (2003) stated that the effect of nisin when combined with sodium lactate have powerful effect against Gram-negative pathogens particularly *E.coli.*

Masschalck et al (2003) reported that nisin has wide spectrum of activity against Gram-positive bacteria including spore-formers but its action is not significant against Gram-negative bacteria In this respect, Gram-positive bacteria are characterized by presence of high contents of anionic lipids in their cell membranes which can be easily penetrated by nisin thus, disruption of the

bacterial cell membrane results in the passage of nisin to the cytoplasmic membrane as the site of nisin action **.**

Gill and Holly (2004) reported that all *E.coli* were highly resistant to the bactericidal effect of nisin.

Hassan et al (2005) stated that the effect of nisin when combined with sodium lactate have powerful effect against Gram-negative pathogens. It is postulated that sodium lactate destabilize the cell membrane of other Gram-negative bacteria by chelating of Mg++ and /Ca++ affecting its permeability to be sensitive to the action of nisin **.**

Belfiore et al (2006) reported that the inhibitory activity of two bacteriocins produced by *Lactobacillus curvatus* CRL705 and nisin (1066AUml_1) produced by *Lactococcus lactis* CRL1109 in combination with chelating agents against *E. coli* strains in TSB medium at 21 and 6 C was investigated. Treatment with EDTA (500 and 1000mM) and Na lactate (800mM) alone produced a variable effect depending on the strain, Na lactate being inhibitory against *E. coli* NCTC12900 at both assayed temperatures while EDTA (1000mM) led to its inactivation only at 6 ^{o}C. Direct and deferred strategies using EDTA and Na lactate showed that the direct addition of bacteriocins and chelators was not as effective as compared to deferred treatments. When the deferred treatment effectiveness was evaluated at 6 C, the use of EDTA (500 and 1000mM) and Na lactate (800mM) in combination with lactocin 705/AL705 demonstrated to be the most inhibitory strategy against both *E. coli* strains. Nevertheless, treatments with chelators and bacteriocins was highly dependent upon strain sensitivity. Permeabilization of the outer membrane of *E. coli* strains with EDTA and Na lactate combined with

lactocin 705/AL705 showed to be valuable in controlling this foodborne bacteria at low temperatures.

Solomakos et al (2008) stated that the treatment of minced beef meat or TSB with nisin at 500 or 1000 IU/g did not show any antibacterial activity against *E. coli* O157:H7. The combination of thyme essential oil at 0.6% and nisin at 500 or 1000 IU/g showed an additive effect against the pathogen, which was higher during storage at 10 ^{o}C than at 4 ^{o}C.

Cheng-An Hwang et al (2011) stated that the effect of Sodium lactate on the survival of *E. coli* O157:H7 in cooked ham during storage at refrigerated and abuse temperatures. Cooked ham was mixed with 0% - 3% lactate, inoculated with *E. coli* O157:H7 and stored at 4°C - 15°C for up to 35 day. The growth of the pathogens was inhibited in ham containing 3% lactate, and no growth of *E. coli* O157:H7 occurred at the lowest storage temperatures of 6 ^{o}C. In ham containing no lactate, the average growth rates was 0.242 - 0.315 log CFU/day for *E. coli* O157:H7 at 8°C - 15°C.The addition of 1% or 2% lactate significantly ($P < 0.05$) reduced the growth rate of the pathogen, and the effect was more profound at lower temperatures.

Pattanayaiyin et al (2014) stated that the effects of lauric arginate (LAE) and nisin Z, alone or in combination, on cell damage were investigated against *Escherichia coli* O157:H7 by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) observations, efflux of potassium and phosphate ions, and growth inhibition. A combination of LAE with nisin Z caused severe and dramatic changes in the cytoplasmic membrane and cell lysis of both Gram-positive and Gram-negative bacteria. The combination treatment also

caused significant potassium and phosphate ion leakage of *E. coli* O157:H7, when compared with other treatments: 16.62±1.05, 50.35±0.81 of potassium ion and 122.66±8.81, 97.96±3.31 of phosphate ion after treatment for 6h. Bacteria were reduced by approximately 7log10CFU/mL within the first hour of treatment and then cells were unable to grow for the remainder of the experiment. Treatment with LAE alone resulted in changes in cellular morphology, coagulation of the cytoplasm, and low level leakage of potassium and phosphate ions in all bacteria tested. The bacteriocin was not effective against *E. coli* O157:H7.

3- Material and methods

3-1-Collection of the samples:-

A grand total of 250 random samples of meat products; 50 each of minced beef, raw kofta, beef burger, fresh sausage and beef luncheon were collected from different supermarkets at different localities . The collected samples were transferred under hygienic conditions in an ice box to the laboratory without undue delay to be examined for isolation and identification of *Escherichia coli* using both conventional method and PCR technique.

3-2-Preparation of the samples (*APHA ,2004):-*

Each sample of the collected meat products was divided into two parts, a part used for isolation and identification of *E. coli* by conventional method and the other was prepared for isolation and identification of *E. coli* using recent PCR technique.Twenty five grams of the examined meat product samples were transferred to 225 ml of sterile buffered peptone water (0.1%) then homogenized by stomacher (Seward stomacher 80 Biomaster , serial No. 46464, England) for 2 minutes to provide a homogenate of 1/10 dilution.

3-3-Isolation and identification of *E.coli*

3-3-1-Conventional Recovery methods (*APHA ,2004):-*

Isolation of *E.coli*

Selective enrichment:

Twenty five grams from each preperaed sample were transferred to 225 ml of trypticase soya broth (TSB), blended with stomacher at medium speed for one minute and incubated at 37 °C for 24 hours.

Selective plating:

Samples were examined by streaking a loopful from each previously incubated enrichment broth over Eosine Methelene Blue agar and Sorbitol MacConkey agar plates (SMAC) supplemented with cefixime and tellurite then the inoculated plates were incubated at 37°C for 24hours .Suspected colonies were purified on slope agar tubes where they incubated at 37°C for 24hours for further identification.

Identification of *E.coli:-*

Morphological examination:-

Staining *(Cruickshank et al., 1975)*:

Films were prepared from the pure cultures of isolated colonies and stained with Gram's stain technique and examined microscopically. Gram negative, coccobacilli medium size rods, stained evenly and non sporulating were subjected to biochemical identification.

Motility test *(ICMSF, 1996)*:

Suspected organisms were inoculated separately into semi-solid agar tubes and incubated at 37 ^{o}C for 24 hours. Motility was evidenced by a circular growth around the line of inoculation. *E.coli* showed negative reaction.

Biochemical identification:

Eijkman test (Cruickshank et al., 1975) :

Five mls of MacConkey's broth tubes provided with inverted Durham's tubes were used. The tubes were kept in a water bath adjusted at 44 ± 0.5°C for 24 hrs before inoculation. A loopful of each suspected pure culture was inoculated into each tube before being incubated at 44 ± 0.5c° for 48 hrs. Production of acid and gas was considered as a positive result.

Indole production test *(Kovac's, 1928):*

To 5 ml peptone water (1 %) culture previously incubated at 37 ^{o}C for 48 hrs, one ml of ethyl ether was added. The tubes was vigorously shaken and allowed to stand for few minutes until the ether rises to the surface. About 0.5 ml of Ehrlich's reagent was gently trickled on the side of the tube, development of rosy ring indicates presence of indole. *E.coli* is indole positive.

Voges-Proskauer test *(Ljutov, 1963)*:

To 5 ml of 48 hours glucose phosphate broth culture incubated at 37 ^{o}C 3 ml alcoholic solution of alpha-naphthol and 1 ml 40% Potassium hydroxide solution were added. The mixture was thoroughly shaken and examined after 15 minutes. A positive reaction was indicated by appearance of bright pink color. *E.coli* showed a negative reaction.

Methyl red test *(Ljutov, 1963)*:-

To 5 ml of 48 hours glucose phosphate broth culture incubated at 37 ^{o}C, 5 drops of methyl red reagent were added. Appearance of red color indicates positive result. *E.coli* is methyl red positive.

Citrate utilization test *(Simmons, 1926):*

A loopful from each pure suspected culture was inoculated into butt and slant of Simmon's citrate agar tubes incubated at 37 ^{o}C for 5 days. Development of blue color indicate positive result. *E.coli* is citrate negative.

Lysin decarboxylase activity *(Macfaddin, 2000):*

An inoculum from 24 hours pure culture of isolated organism was transferred into a tube of lysin broth. The inoculated tubes as well as control containing no lysin were incubated at 35 ^{o}C for at least 24 hours and up to 4 days, after the first 12 hours of incubation the colour of the indicator changed from purple to yellow.The return of purple color after further incubation is considered as positive reaction.*E.coli* gives positive reaction.

Sugar fermentation *(Macfaddin, 2000):-*

A loopful from each suspected culture was inoculated into 1% peptone water tube and 0.2 % bromocresol purple as indicator . One % of the following sugars (glucose, lactose, mannitol, dulicitol, salicin, sorbitol and insitol) was added to each tube before being inoculated and incubated at 37 ^{o}C. The reaction was noticed every 24 hours for 7 successive days and recorded.

Hydrolysis of urea *(Christensen, 1946)*:

The suspected isolates were stabbed into the butt and streaked onto the slant of the urea agar tubes. The inoculated tubes were incubated at 37 ^{o}C and examined after 4 hours then examined daily for 5 days. Development of red colour indicates urea hydrolysis. *E.coli* showed positive results.

H2S production test (Macfaddin, 2000) :

The suspected pure culture was stabbed into the butt of Triple Sugar Iron Agar (TSI) tubes, then streaked onto the slant. The tubes were incubated at 37°C and examined daily up to 7 days. Production of black color due to H2S production and/or gas production were recorded.

Vero cell assay of the suspected E.coli strains

The cytotoxicity of the suspected *E.coli* isolates for vero cells was determined by using tissue culture superntant and there by detecting only high level of producers of these cytotoxins based on ***Konowalchuk et al. (1977).***

Preparation of chemicals for vero cytotoxicity assay

HEPES buffer:

For preparation of one liter of stock solution of HEPES (10X)

NaCl…….. 40.0 g.

KCl…….. 1.5 g.

HEPES……. 11.9 g.

Glucose…… 10.0 g.

These chemicals were dissolved in 450 ml distilled water and the PH was adjusted to 7.55 by using 4 N NaOH, then completed up 1 liter with distilled water and sterilized by filtration. Working (1X) solution was prepared by adding of 450 ml sterile distilled water to 50 ml stock of HEPES solution.

RPMI 1460 medium:

Two table spoonful of active charcoal were allowed to dissolve in 10 liters of distilled water overnight by using magnetic stirrer, and then the solution was left for one hour to allow the precipitation of undissolved charcoal. The supernatant was filtered through 0.1 µm filter. 104.3 g of RPMI 1460 (Gibco Co.) were added to the filtrated supernatant with 2 g/1 $NaHCO_3$. This mixture was subjected to stirring for 3 hours and then filtered through 0.1 µm filter.

Phosphate buffered solution (PBS).

For preparation of one liter stock solution of PBS (10X)

NaCl…….. 100 gr.

KCl……. 5.0 gr.

KH_2PO_4…… . 2.5 gr.

$NaHPO_4$ 2H2O…… 180 gr.

$NaHPO_4$ $2H_2O$ was dissolved at first in distilled water and then the other chemicals were added and dissolved. The solution was sterilized by filtration.

Minimal medium:

For preparation of one liter stock solution of minimal medium (10 X) the following chemicals were dissolved in 100 ml sterile distilled water:

K_2HPO_4…… 3.5 gr.

KH_2PO_4……1.5 gr.

C_6H_5 Na $2H_2O$……0.5 gr

Mg SO_4……0.1 gr.

$(Na4)_2$ SO_4……..1.0 gr

Glucose……2.0 gr

Trimethoprin……20.0gr

Sulphametoxazol……100 gr.

Cell culture medium:

For preparation of 0.5 liter of the medium

1% glutamine……..5ml

1% penicillin/streptomycin……..5ml

10% fetal calf serum……50ml

0.15 mg oxalate/ml……75 mg

The solution was completed until 500ml with distilled water and the pH adjusted to 7-7.2 and then sterilized by filteration.

EDTA 0.2% solution:

0.2 g. EDTA sodium was dissolved in 100 ml sterile HEPS solution (1X).

Preparation of the bacterial lysates:-

For detection of shiga-like toxin producing *E.coli* (SLTEC), single colonies were inoculated into 15 ml trypticase soya broth tubes before being incubated for 6 hours at 37 ^{o}C. After incubation the broth tubes were spilled into 50 ml sterile tubes, filled with 20 ml minimal medium and incubated overnight at 37 ^{o}C with shaking. In the early morning the tubes were centrifuged at 4000 rpm/min. for 30 minutes at 4 ^{o}C. The supernatant was discarded and the pellets were resuspended in 2 ml polymyxin B-PBS (0.1mg/ml) and incubated at 37^{o}C for 30 minutes with shaking. Each sample was transferred into two sterile Eppendorf tubes which were centrifuged at 10000 rpm/min., 4 ^{o}C. The supernatant was then transferred into fresh sterile Eppendorf tubes and was frozen at -20 ^{o}C until be used.

Vero cell culture

Vero cells obtained from **Vac. Sera, Doki, Egypt** were grown and maintained in cell culture flasks at 37^{o}C in 5% CO2 atmosphere. The cells were supplmnted with RPMI 1640 medium, 10% fetal calf serum, 2mM L-glutamin, 100 U penicillin/ml and 100 µg streptomycin /ml. These cells were rinsed with 10 ml sterile HEPES solution after removing the old medium and incubated for 5 minutes, then rinsed with 5 ml 0.2% EDTA in PBS and 5 ml of 0.25% trypsin in PBS and incubated for 10 minutes. After detaching the cells from the surface of the tissue culture flask, they were transferred to a sterile tube and centrifuged at 500 rpm/min. for 5 mintes. The supernatant fluid was discarded and the formed pellet was resuspeded in 10 ml RPMI.

Test method:-

The cytotoxic assay was carried out after modification by **Gentry and Dalrymple(1980).**

The test was carried out in 96 well tissue culture plates. 90µL of sterile physiological saline was added to each of the test wells ,while 50µL of the physiological saline was added to the negative control wells. 60 µL of the bacterial lysates was added to each well. 50µL of RPMI medium containing 10% calf serum**,** 2mM L-glutamin, 100 U penicillin/ml and 100 µg streptomycin /ml were added to each one of the test wells**.** A suspension of vero cells was prepared and 50 µL of this suspension was seeded in each well of the test wells. 50 µL of 1% SDS solution was added to each of the positive control wells. The plates were incubated at 37^{o}C in 5% CO2 atmosphere, observed daily by using inverted microscope for

detection of cell lysis and vacuolation as compared with control test and after four days .

Serological identification of isolated *E. coli.*

The obtained isolates were serologically identified according to ***Kok et al.*** **(1996)** by using rapid diagnostic *E.coli* antisera sets (**DENKA SEIKEN Co., Japan**) for diagnosis of the Enteropathogenic types of *E.coli* where, two separate drops of saline were put on a glass slide and a portion of the colony from the suspected culture was emulsified with the saline solution to give a smooth fairly dense suspension. To one suspension, control, one loopful of saline was added and mixed. To the other suspension one loopful of undiluted antiserum was added and titled back and forward for one minute. Agglutination was observed using indirect lighting over a dark background. When a colony gave a strongly positive agglutination with one of the pools of polyvalent serum, a further portion of it was inoculated onto a nutrient agar slant and incubated at 37°C for 24 hours to grow as a culture for testing with mono-valent sera.A heavy suspension of bacteria from each slope culture was prepared in saline, and slide agglutination tests were performed with the diagnostic sera to identify the O-antigen.

The diagnostic *E.coli* antisera sets used for identification include the following sets:

Set 1 : O- antisera:

Polyvalent antisera 1: O1, O26, O86a, O111, O119, O127a and O128.

Polyvalent antisera 2: O44, O55, O125, O126, O146 and O166.

Polyvalent antisera 3: O18, O114, O142, O151, O157 and O158.

Polyvalent antisera 4: O6, O27, O78, O148, O159 and O168.

Polyvalent antisera 5: O20, O25, O63, O153 and O167.

Polyvalent antisera 6: O8, O15, O115 and O169.

Polyvalent antisera 7: O28ac, O112ac, O124, O136 and O144.

Polyvalent antisera 8: O29, O143, O152 and O164.

Detection of Stx1, Stx2 , eae and Hemolysin (*hlyA*) genes in some *E.coli* strains isolated from meat product samples using Multiplex PCR: (Paton and Paton,1998)

The multiplex PCR was performed at the laboratory of infectious diseases and internal medicine, Faculty of veterinary medicine, Sadat City University.

Materials used for Multiplex PCR.

- **Genomic DNA extraction:**

DN Using GeneJET Genomic DNA Purification Kit

Lysis buffer

Lysis solution

Protinase K

RNase

50% ethanol

Wash Buffer I

Wash Buffer II

- **DNA amplified products:**

PCR master Mix (Fermentis)

PCR buffer

2.5mM MgCl2

200 mM each the four deoxynucleoside triphosphates d.ATP.

d.CTP. dGTP and dTTP

2.5 U of Taq DNA polymerase

- **Primer sequences and predicted lengths of multiplex-PCR amplification products: (Sigma pioneer) according to Paton and Paton(1998)**

Target gene	Direction	Primer sequence (5`-3`)	Fragment size (bp)
Stx1	Forward	ATAAATCGCCATTCGTTGACTAC	180
	Reverse	AGAACGCCCACTGAGATCATC	
Stx2	Forward	GGCACTGTCTGAAACTGCTCC	255
	Reverse	TCGCCAGTTATCTGACATTCTG	
hlyA	Forward	GCATCACAAGCGTACGTTCC	534
	Reverse	AATGAGCCAAGCTGGTTAAGCT	
eae	Forward	GACCCGGCACAAGCATAAGC	384
	Reverse	CCACCTGCAGCAACAAGAGG	

- **Gel Elecrophoresis: Sambrook et al. (1989).**

a- Agarose

b- Ethidium bromide

c- Tris, boric acid EDTA (TBE) 10x

Tris base (1 M)	121gm
Boric acid (1M)	51.3 gm
EDTA 2H2O	3.72 gm
Water to one litre	

Genomic DNA extraction:

Using GeneJET Genomic DNA Purification Kit (Fermentas)

One ml of an overnight incubated broth was centrifuged at 13000 rpm for 2 minutes at 4C^{o} and the supernatant was discarded.

The pellet resuspended in 180 ul of Gram positive bacteria lysis buffer which consists of 20mM Tris-Hcl, 2mM EDTA, 1.2% Triton X-100 and lysozyme 20mg/ml. and then incubated for 30 min at 37^{o}C.

200ul of lysis solution and 20ul of protinase K were added and mixed thoroughly by vortexing to obtain uniform suspension.

The sample was incubated at 56^{o}C with periodical vortexing until the cells are completely lysed (30 min).

20ul of RNase A solution were added, mixed by vortexing and the mixture incubated for 10 min at room temperature.

400 ul of 50% ethanol were added and mixed by vortexing.

The prepared lysate was transferred to GeneJET Genomic DNA Purification Column inserted in a collection tube. The column was centrifuged for 1 min at 6000xg. The collection tube containing the flow-through solution was discarded. The GeneJET Genomic DNA Purification Column was Placed into a new 2 ml collection tube (included).

500ul of Wash Buffer I (with ethanol) were added and centrifuged for 1 min at 8000xg. The flow-through discarded and the purification column placed back into the collection tube.

500ul of Wash Buffer II (with ethanol) were added to the GeneJET Genomic DNA Purification Column and centrifuged for 3 min at maximum speed (12000xg).

The collection tube containing the flow-through solution discarded and the GeneJET Genomic DNA Purification Column transferred to a sterile 1.5 ml microcentrifuge tube.

200 ul of Elution Buffer were added to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA . incubated for 2 min. at room temperature and centrifuged for 1 min at 8000xg.

The purification column discarded. The purification DNA used immediately in down stream application or stored at -20^{o}C.

DNA amplification for Multiplex-PCR reaction.

20ng of chromosomal DNA was used per reaction , where amplifications were performed in 25ul of buffer solution containing 3uM of oligonucleotides, 200uM of each deoxynucleoside triphosphate, 3.5 mM $MgCl_2$ and 2.5U of DNA Taq polymerase.

Mixtures were overlaid with mineral oil and amplification was performed in PCR thermal cycler. Samples were subjected to 35 PCR cycles, each consisting of 1 min of denaturation at 95°C; 2 min of annealing at 65°C for the first 10 cycles, decrementing to 60°C by cycle 15; and 1.5 min of elongation at 72°C,incrementing to 2.5 min from cycles 25 to 35. Amplified DNA fragments were resolved by gel electrophoresis (**Sambrook et al, 1989**) using 2 % (w/v) agarose. Gels were stained with 0.5 mg of ethidium bromide per ml for 15 min, and documented with a UVP documentation system.

Detection of amplified products:

Thirty µl from each PCR amplified products were electrophoresed on agarose gel, stained with ethidium bromide solution (0.5 µg/ml), visualized under an ultraviolet transilluminator and photographed. Visible bands of appropriate size of 180 bp (*Stx1*), 255 bp (*Stx2*), 384 bp (*eae*) and 534 bp (hlyA) were considered positive. Molecular mass markers (100 bp ladder) were electrophoresed simultaneously.

3.3.2 Rapid method using PCR for detection *E. coli* in beef meat products.(Daly et al., 2002).

- **Preparation of samples:**

25gm of each prepared sample was added to 225 of brain heart infusion broth and stomached in stomacher (Seward stomacher 80 Biomaster , serial No. 46464. England) for 2 minutes then incubated over night.

- **Genomic DNA extraction:**

One ml of an overnight incubated broth was centrifuged at 13000 rpm for 2 minutes at 4c^{o} and the sediment was suspended in equal volume of Tris-EDTA buffer. Furthermore, 100µl of lysozyme solution (10 mg/L), 100 µl of protienase K enzyme (0.3 mg/L) and 1% dodecyl sulphate were added.

The DNA lysate was extracted once with chloroform/isoamyl alcohol (24:1, ratio by volume), then extracted with phenol/ chloroform/isoamyl alcohol (25:24:1, ratio by volume). The aqueous phase was mixed with isopropanol alcohol and incubated at -20C for 30 minutes. The precipitated DNA was spooled out, rinsed in 70 % ethanol and dissolved in 0.5 ml of Tris EDTA buffer.

- **DNA amplification for PCR reaction:**

- The PCR reaction mix (50 µL) for each sample was consistent of:

10 µL extracted DNA

2.5 µL primers mix.

Oligonucleotide primers for PCR amplification.

Target gene	Direction	Primer sequence (5`-3`)	Fragment size (bp)	Reference

E.coli	Forward	CTGGAAGAGGCTAGCCTGGACGAG	366	**Yokoigawa et al. (1999)**
	Reverse	AAAATCGGCACCGGTGGAGCGATC		

1 µL deoxynucleoside triphosphate (dNTP-mix)

5 µL 10x buffer

1 µL Taq-DNA polymerase enzyme (5000 U/ml)

30.5 µL ultra pure deionized water.

The reaction mixture was overlaid with mineral oil and was incubated in the thermal cycler as follows:

- The first initial cycle: 95 C for 6 minute (denaturation), 35C for 2 minutes (annealing) and 72 C for 1.5 minutes (extension).
- The consequent 35cycles:95C for 20 seconds (denturation), 35 C for one minute (annealing) and 72 C for 1 minutes (extension).
- The final extension step at 72 C for 5 minutes then kept at 4C (hold teprature).

- **separation of PCR amplicons by Gel Electrophoresis:**

After the amplification was completed the amplified products was analyzed on agarose gel (consisted of 2% agarose and 5 µL of ethidium bromide in 1 x Tris –Acetate EDTA (TAE) buffer. The samples were electrophoresed at 100

volts for one hour, shown under ultra violet transiluminator and photographed. Visible bands of appropriate size of 366 bp were considered positive.

3-4-Biocontrol of *E.coli* in beef products by using of Nisin alone or combined with other chemical preservatives.

Preperation of minced meat

A total of 96 frozen beef samples (50 g each) were minced, packaged and divided into 4 groups (24 each). Each group was subdivided into 3 classes to study effect of nicin, either alone or combined with other chemical preservatives, on the viability of *E.coli* O111:H4 in tested samples.

Incoulation of minced meat samples with *E.coli* O111:H4.

All tested minced beef samples proved to be free from *E.coli* by using conventional isolation of *E.coli* were evenly inoculated with *E.coli* O111:H4 strain and suspended in peptone water by an intensity 1×10^6 organisms per each gram.The Population of investigated pathogen was evaluated in peptone water by MacFarland,s nephelometer standards according to **Finegold et al.(1978)** , and confirmed in inoculated meat samples by enumeration technique on plates of eosin methyelne blue(EMB) medium.

Addition of nisin and other chemical preservatives to the inoculated minced beef samples:

The four different formulae were thoroughly mixed with the tested minced beef samples after being inoculated with *E.coli* O111:H4 for demonstrating the destructive action of nisin and other chemical preservatives (one formula for each samples group):

Nisin alone was used by concentration of 10 and 30 ppm (one concentration for each sample class).

Each of 10 ppm and 30 ppm of nisin concentration combined with 0.2% sodium acetate (one concentration for each sample class).

Each of 10 ppm and 30 ppm of nisin concentration combined with 0.2% potassium sorbate (one concentration for each sample class).

Each of 10 ppm and 30 ppm of nisin concentration combined with

0. 1% sodium lactate (one concentration for each sample class).

The inoculated and treated minced beef samples were kept refrigerated at + 4 for different duration (12, 24 and 48 hours).

Enumeration of *E.coli* O111 in minced beef samples:

Ten grams were taken 4 times from each sample; once after inoculation and before adding nisin, while the remaining 3 time were taken after 12, 24 and 48 hours from adding nisin and sodium lactate formula. Every 10 g of minced beef sample were homogenized with 90 ml of buffered peptone water and ten – fold serial dilutions were then made **(ICMSF, 1996).** From each dilution , 0.1 ml was spread onto the dried surface of duplicated plates of eosin methyelne blue (EMB) medium which incubated at $37C^0$ for 24 hours. The growing colonies, having green metallic shine, were enumerated and the initial population as well as the survivors of organism per each gram of tested meat samples were then calculated.

Statistical analysis:

Statistical evaluation including analysis of variance (ANOVA) was used. **Rosner (2002)**

4. RESULTS

Table (1): Incidence of *E. coli* in the examined meat product samples using conventional method and PCR technique (n=50)

Meat products	Conventional method		PCR technique	
	No	%	No	%

Minced meat	23	46	19	38
Beef burger	14	28	11	22
Beef sausage	16	32	15	30
Beef kofta	18	36	16	32
Beef luncheon	8	16	6	12
Total	79	31.6	67	26.8

Figure (1): Incidence of *E. coli* in the examined meat product samples using conventional method and PCR technique.

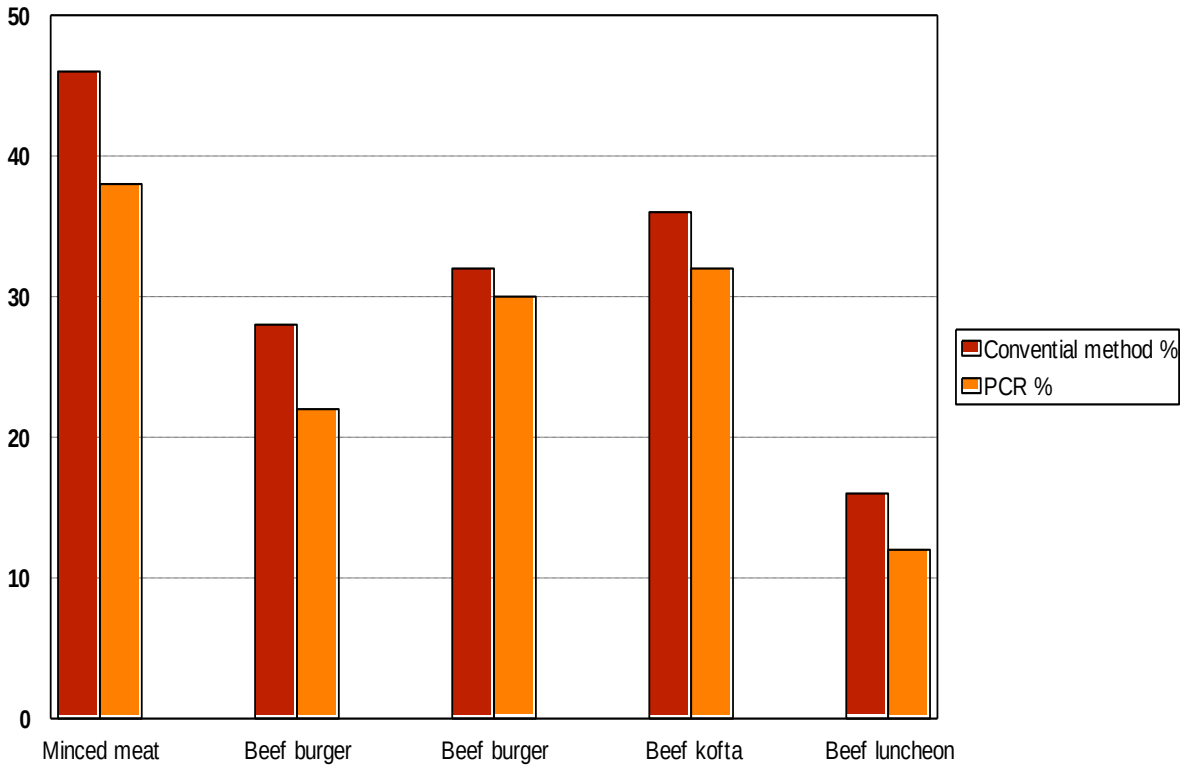
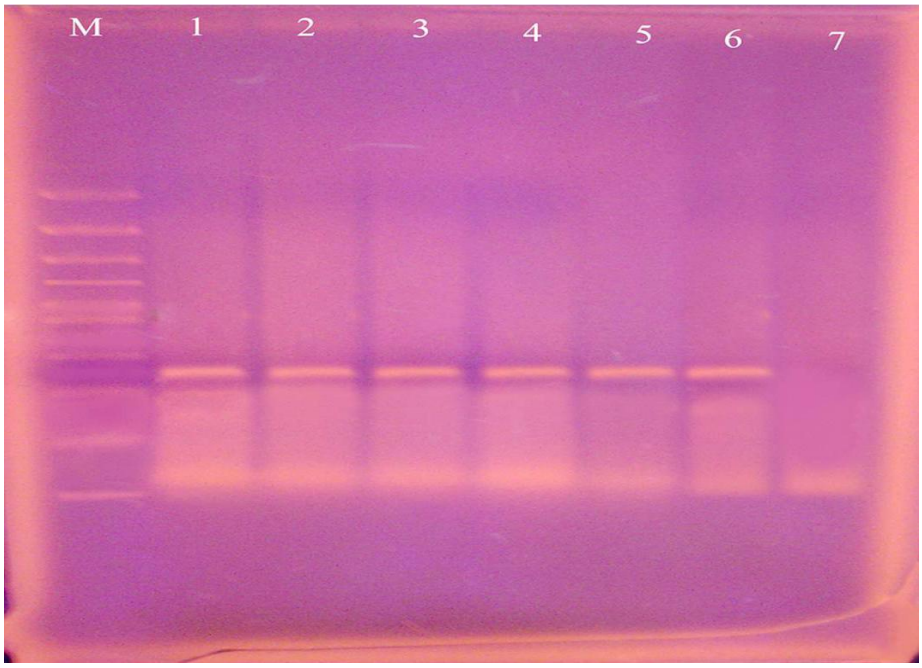


Photo (1) Agarose gel showing five positive samples contaminated with *E. coli* using PCR.



366 bp→

Lane M: MW marker = 100 bp DNA ladder (Promega)

Lane 1- postive control

Lane 2, 3, 4, 5, 6 show several bands at level of 366 bp.

Lane 7: Negative control

Table (2): Incidence of Shiga toxin producing *E. coli* (STEC) in the examined meat product samples using Vero-Cytotoxicty Assay (VCA) (Relation to total number of positive *E. coli* samples).

Meat products	No. of *E.coli* +ve samples	Positive samples	
		No	%
Minced meat	23	14	60.8
Beef burger	14	7	50
Beef sausage	16	7	43.7
Beef kofta	18	9	50
Beef luncheon	8	1	12.5

Figure (2): Incidence of Shiga toxin producing *E. coli* (STEC) in the examined meat product samples using Vero-Cytotoxicty Assay.

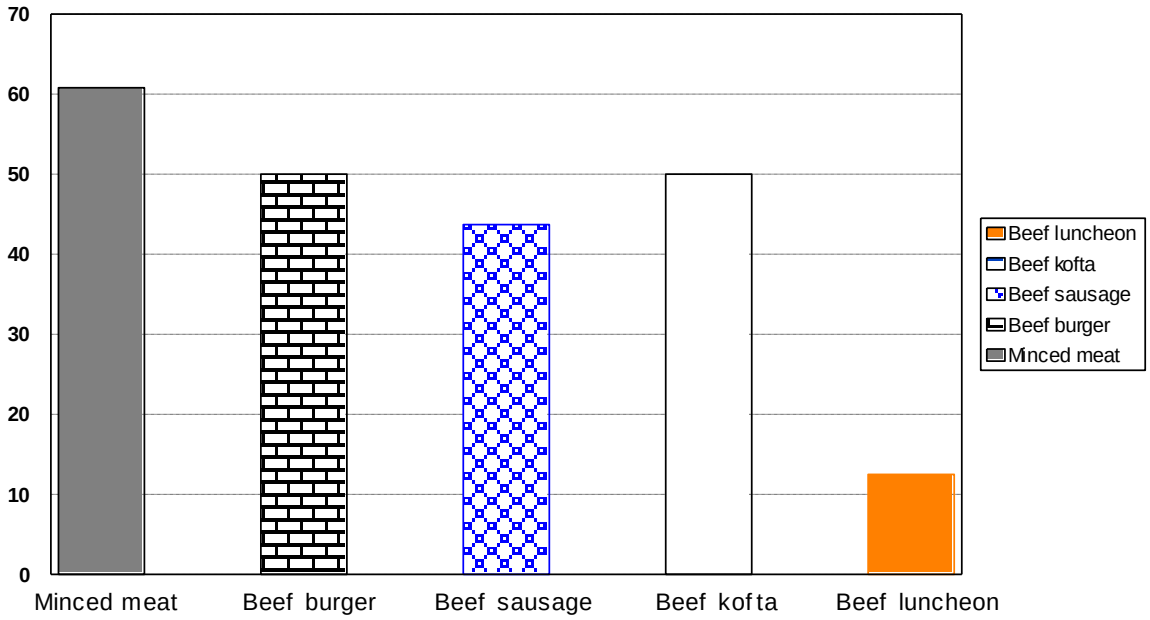


Photo (2): Intact vero cell monolayer incubated with control medium

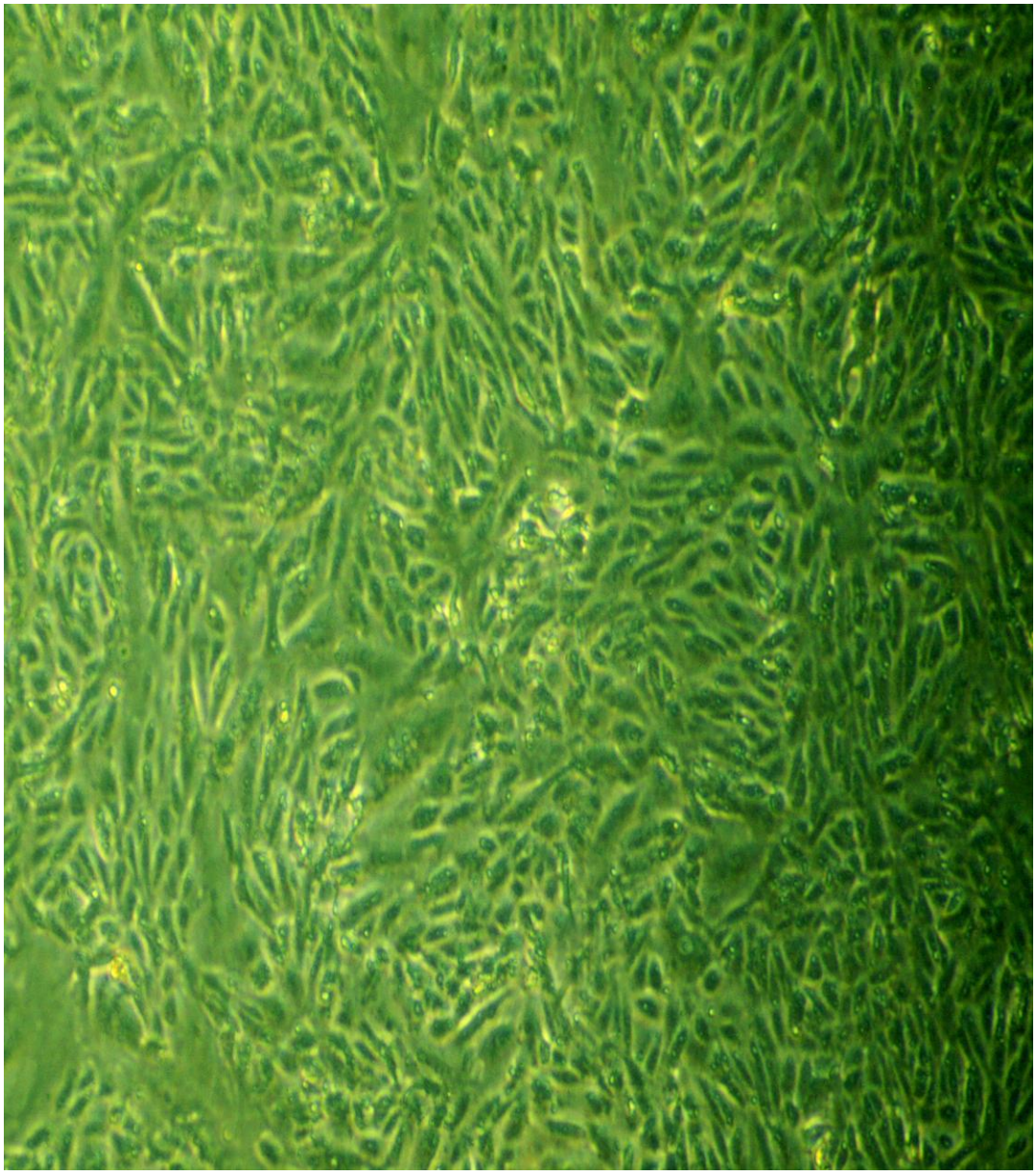
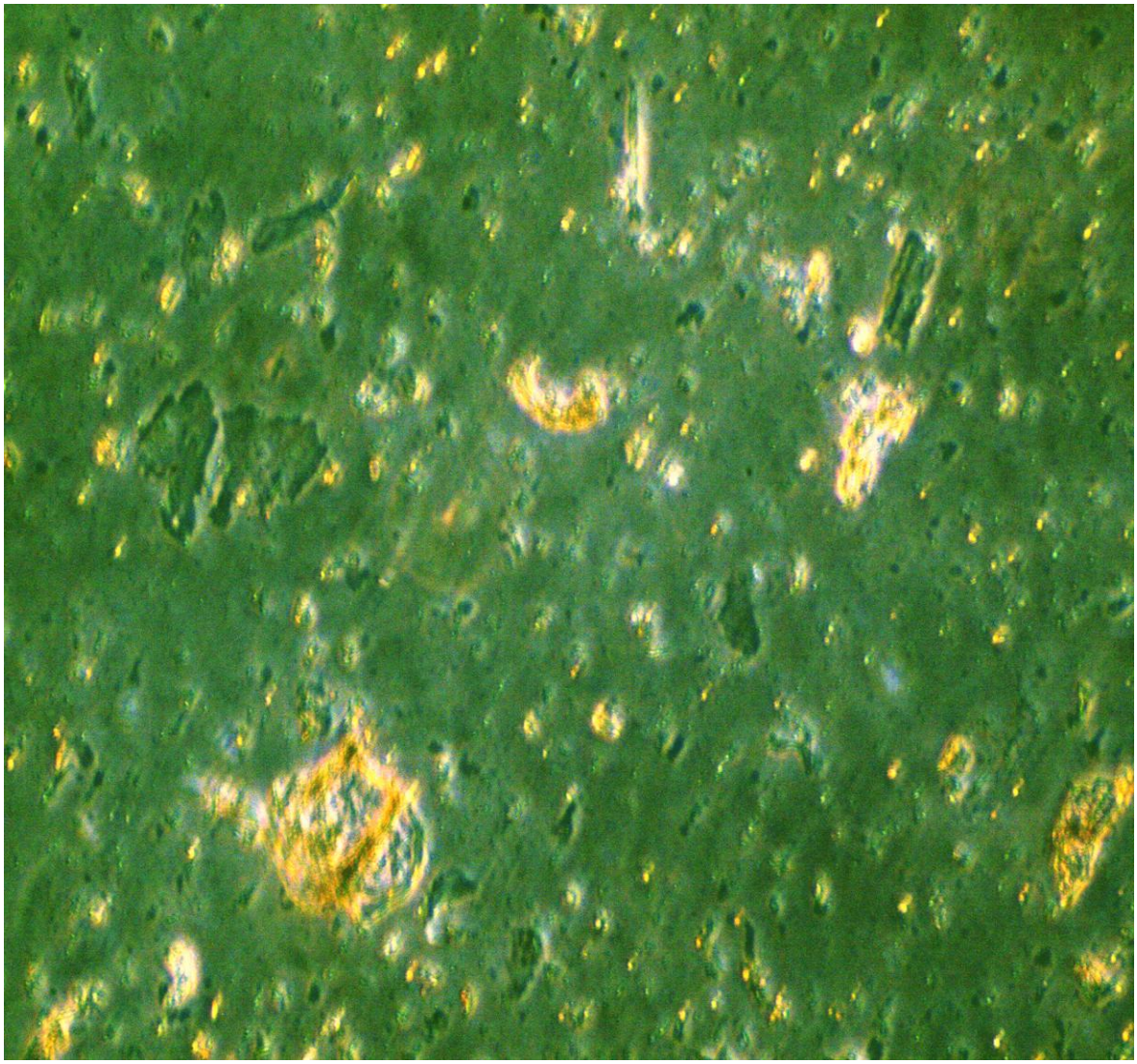


Photo (3): Cytotoxic effect of Shiga toxin containing bacterial lysate of STEC on Vero cells



The Cytopathic effects Shiga toxin containing bacterial lysate of STEC were observed after incubation with culture filtrates there was a change from spindle-

shaped cells characteristic of normal Vero cells to round and shriveled cells, and these changes were followed by gradual destruction of the monolayer.

Table (3): Incidence of Shiga toxin producing *E. coli* (STEC) in the examined meat product samples using Multiplex PCR (Relation to total number of VCA positive samples).

Meat products	No.of VCA+ve samples	No. of PCR +ve samples	%
Minced meat	14	11	78.57%
Beef burger	7	6	85.71%
Beef sausage	7	5	71.42%
Beef kofta	9	8	88.88%
Beef luncheon	1	1	100%

Total	38	31	81.57%

The overall sensitivity of polymerase chain reaction (PCR) = 81.57% compared with Vero Cytotoxicity Assay for detection of Shiga toxin-producing *E.coli* (*Stx1*, S*tx2* or both) in meat product samples.

Table (4): Incidence of Shiga toxin producing *E. coli* (STEC) serovars isolated from examined meat product samples.

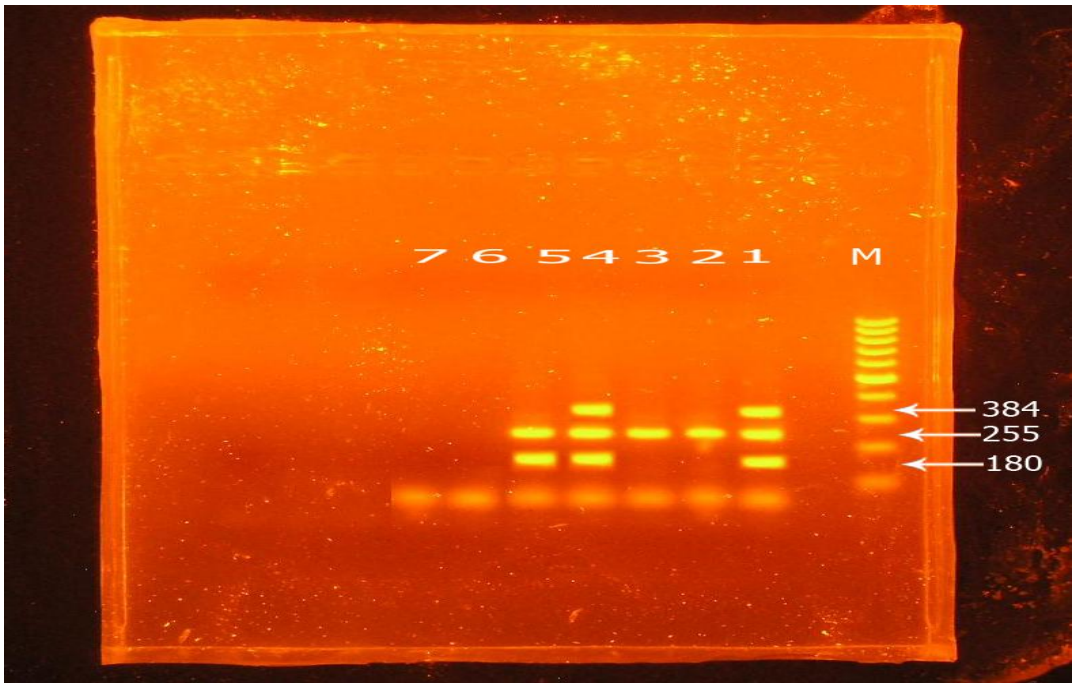
E.coli Serovars	Meat products				
	Minced meat	Beef burger	Beef sausage	Beef kofta	Beef luncheon
O111	3	1	1	2	--
O26	1	1	--	1	--
O103	1	1	1	--	--
O91	--	--	--	1	--
O119	1	--	1	--	--
O128	2	--	--	2	1
O86	1	1	1	1	--
O146	1	1	--	1	--
O45	--	1	1	--	--
O121	1	--	--	--	--
Total	11	7	7	9	1

Table (5): Occurrence of virulence genes of Shiga toxin-producing *E.coli* (STEC) isolated from examined meat product samples.

Serovars	No. of ex. isolates	Stx1 alone		Stx2 alone		Stx1&Stx2		Eae	
		NO.	%	NO.	%	No.	%	No.	%
O111	7	6	85.7	7	100	6	85.7	5	71.4
O26	3	2	66.6	3	100	2	66.6	2	66.6
O103	3	1	33.3	2	66.6	2	66.6	0.0	0.0
O91	1	1	100	1	100	1	100	0.0	0.0
O119	2	1	50	2	100	1	50	0.0	0.0
O128	5	5	100	0.0	0.0	0.0	0.0	0.0	0.0

O86	4	**0.0**	**0.0**	**4**	**100**	**0.0**	**0.0**	**0.0**	**0.0**
O146	3	**0.0**	**0.0**	**3**	**100**	**0.0**	**0.0**	**0.0**	**0.0**
O45	2	**2**	**100**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
O121	1	**0.0**	**0.0**	**1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Photo (4) Agarose gel shows five positive strains of EHEC for shiga toxin 1 and shiga toxin 2 and eae genes (180 bp, 255bp, 384 bp) respectively.



Lane (M): MW marker = 100 bp DNA ladder (Promega).

Lane 1- O_{111} has the 3 genes stx1, stx2 and eae genes

Lane 2- O_{86} has stx2 genes

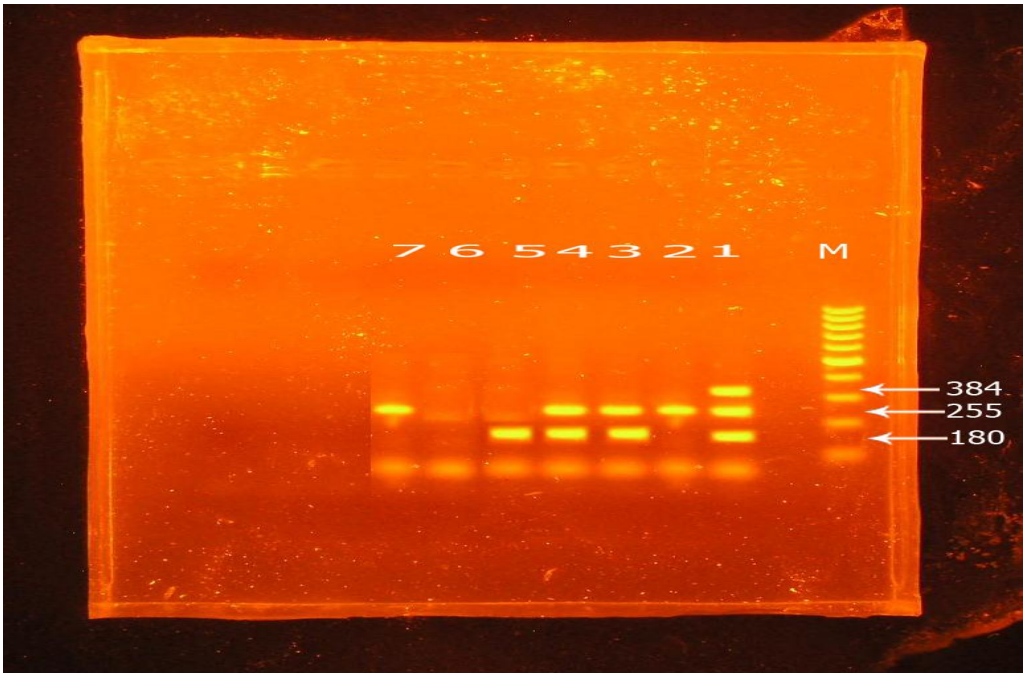
Lane 3- O_{121} has stx2 genes

Lane 4- O_{26} has the 3 genes stx1, stx2 and eae genes

Lane 5- O_{103} harbor stx1 and stx2genes

Lane 6&7: Negative control.

Photo (5) Agarose gel shows six positive strains of EHEC for shiga toxin 1 and shiga toxin 2 and eae genes (180 bp, 255bp, 384 bp) respectively.



Lane (M): MW marker = 100 bp DNA ladder (Promega).

Lane 1: O_{111} has the 3 genes stx1, stx2 and eae genes

Lane 2: O_{146} harbor stx2 genes

Lane 3P: O_{91} harbor stx1 and stx2genes

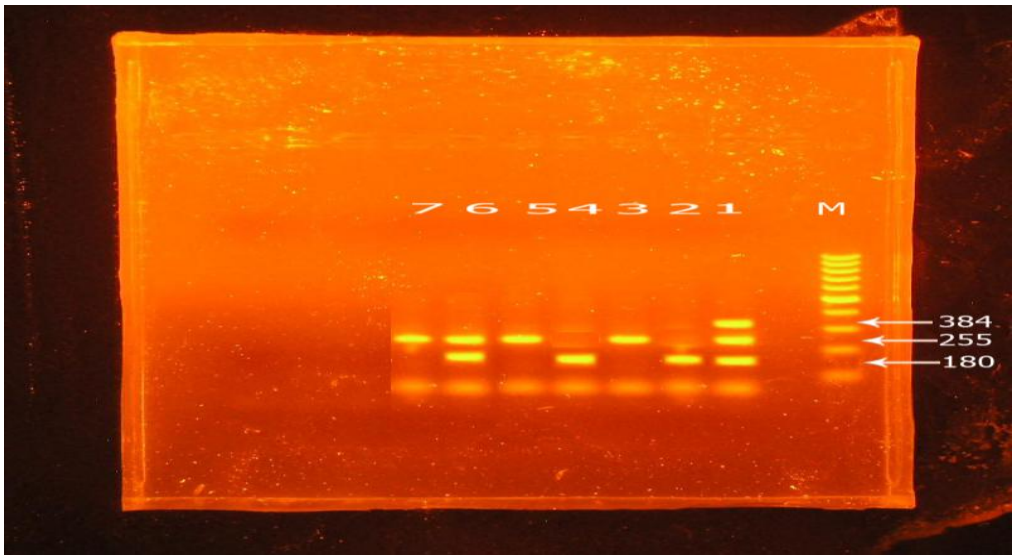
Lane 4: O_{103} harbor stx1 and stx2genes

Lane 5P: O_{45} has stx1 genes

Lane 6: Negative control.

Lane 7: O_{86} has stx2 genes

Photo (6) Agarose gel shows seven positive strains of EHEC for shiga toxin 1 and shiga toxin 2 and eae genes (180 bp, 255bp, 384 bp) respectively.



Lane (M): MW marker = 100 bp DNA ladder (Promega).

Lane 1: O_{111} has the 3 genes stx1, stx2 and eae genes

Lane 2: O_{128} harbor stx1 genes

Lane 3: O_{146} harbor stx2 genes

Lane 4: O_{45} harbor stx1 genes

Lane 5: O_{86} has stx2 genes

Lane 6: O_{119} harbor stx1 and stx2genes

Lane 7: O_{86} has stx2 genes

Table (6): Acceptability of the examined meat product samples according to the EOSQ (2005/1694) according to *E.coli*.

Meat products	EOSQ	Permissible limit	Acceptability	
			No.	%
Minced beef	**2005/1694**	free	27	54

Beef burger	**2005/1688**	free	36	72
Beef sausage	**2005/1972**	free	34	68
Beef kofta	**2005/1694**	free	32	64
Beef luncheon	**2005/1114**	free	41	82

EOSQ = Egyptian Organization for Standardization and Quality Control (2005)

Table (7): Biocontrol effect of different nisin concentrations on *E.coli* O111:H4 inoculated into minced beef samples by an intensity of 1×10^6/g.

Concentration / Duration	10 ppm Nisin			30 ppm Nisin		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
12 hours	9.0×10^3	4.2×10^4	$2.2\times10^4\pm9.9\times10^3$	2.0×10^3	1.0×10^4	$5.6\times10^3\pm2.3\times10^3$
24 hours	4.0×10^3	1.3×10^4	$7.3\times10^3\pm2.8\times10^3$	8.0×10^2	6.0×10^3	$2.6\times10^3\pm1.7\times10^3$
48 hours	2.5×10^3	1.0×10^4	$5.2\times10^3\pm2.3\times10^3$	5.0×10^2	3.0×10^3	$1.4\times10^3\pm7.7\times10^2$

Table (8): Biocontrol effect of different nisin concentrations combined with 0.2% Sodium acetate on *E.coli* O111:H4 inoculated into minced beef samples by an intensity of 1×10^6/g.

Concentration / Duration	10 ppm Nisin + 2% Sod. acetate			30 ppm Nisin+ 2% Sod. acetate		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
12 hours	5.0×10^{3}	3.0×10^{4}	$1.4\times10^{4}\pm7.9\times10^{3}$	2.0×10^{3}	5.5×10^{3}	$2.9\times10^{3}\pm1.3\times10^{3}$
24 hours	2.0×10^{3}	1×10^{4}	$5.0\times10^{3}\pm2.5\times10^{3}$	1.0×10^{2}	3.3×10^{3}	$1.3\times10^{3}\pm9.3\times10^{3}$
48 hours	5×10^{2}	3.4×10^{3}	$2.1\times10^{3}\pm8.6\times10^{2}$	2.0×10^{2}	1.0×10^{3}	$4.4\times10^{2}\pm2.9\times10^{2}$

Table (9): Biocontrol effect of different nisin concentrations combined with 0.2% Potassium sorbate on *E.coli* O111:H4 inoculated into minced beef samples by an intensity of 1×10^6/g.

Concentration / Duration	10 ppm Nisin + 2% Pot. sorbate			30 ppm Nisin+ 2% Pot. sorbate		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
12 hours	1.8×10^3	3.2×10^4	$1.3\times10^4\pm95\times10^3$	7.4×10^2	3.2×10^3	$2.3\times10^3\pm9.7\times10^2$
24 hours	2.4×10^2	1.1×10^3	$6.1\times10^2\pm2.5\times10^2$	5.0×10^2	8.0×10^2	$3.2\times10^2\pm2.4\times10^2$

48 hours	2.0×10^2	9.0×10^2	$5.4\times10^2\pm2.0\times10^2$	2.0×10	1.0×10^2	$6.6\times10\pm2.4\times10$

Table (10): Biocontrol effect of different nisin concentrations combined with 0.2 % Sodium lactate on *E.coli* O111:H4 inoculated into minced beef samples by an intensity of 1×10^6/g.

Concentration / Duration	10 ppm Nisin + 2% Sod. lactate			30 ppm Nisin+ 2% Sod. lactate		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
12 hours	1.0×10^2	8.0×10^2	$4.0\times10^2\pm2.0\times10^2$	6.0×10	3.0×10^2	15×10±7.5×10
24 hours	-----	-----	-----	-----	-----	-----
48 hours	-----	-----	-----	-----	------	------

5-Discussion

Every treatment done to the meat from the point of slaughtering until it is ready for consumption can add to the bacterial load of this meat. Thus, meat products are considered as a major vehicle of most reported foodborne outbreak, and may be contaminated with several types of organisms through long chain of preparation, handling of raw meat, , processing, distribution , storage and retailing. Consequently ,we are in need to apply rapid and accurate methods for detection of such microorganisms as using PCR techniques, and also, Establishing appropriate control methods as using bioperservation.

E. coli in meat products:

The presence of *E. coli* in raw food of animal origin can be expected because of the close association of this food with the animal environment and contamination of the carcass from fecal material, hide during slaughtering and dressing procedures. These organisms are destroyed by heat processing of foods. Thus, the

presence of *E. coli* in a heat processed food means either process failure or more commonly, post processing contamination from equipment, employees or from contact with contaminated raw foods. **(National Academy of Sciences, 1985)**.

The results recorded in table (1) and Fig.(1) revealed that 46%, 28%, 32% , 36% and 16% of examined minced meat, beef burger, beef sausage, beef kofta and beef luncheon samples , respectively were contaminated with *E. coli* using conventional method These results were agreed, to some extent, with these reported by **Gobran (1985), Morshdy (1985) , Nashed-Heba *et al* (1993)** ,**Abou-Hussien- Reham (2004) and Hassan (2012).** Lower results were detected by **Zaki - Eman (1990), Ahmed (1992)** , **El-Feky (1994)**, **Fathi and Thabet (2001)**, **Ouf - Jehan(2001) and Eleiwa - Nesreen (2003)**.

The presence of *E.coli* in food is considered as indicator of faults during preparation, handling, storage or service. It is also, considered as indicator of fecal contamination , besides , it may induce severe diarrhea in infants and young children, as well as food poisoning and gastroenteritis among the adults. *E. coli* was previously were also isolated from meat products by **Caserio and Pantano (1980), Hefnawy (1980), Gouda (1991) and Mousa et al. (1993), and Hassan (2007).**

Table (1)and fig.(1) reported that *E. coli* can be detected by direct PCR technique in the examined minced meat, beef burger, beef sausage, beef kofta and beef luncheon by the following percentages 38%, 22%, 30%, 32% and 12% respectively. These results were agreed with that obtained by **Stampi-Serena (2004) and Hassan (2012)** who detected *E.coli* in 30.2%of tested meat product samples especially in minced meat and burger using PCR technique. Lower incidence could be detected by **Abongo and Momba (2009)** who isolated E. *coli* from 2.8% of the examined meat product samples and **Lee et al. (2009)** who stated that the incidence of *E.coli* was 4.1% in the examined beef samples.

From the previous results , one can conclud that the recovery of *E.coli* was more achieved by using PCR as detection of such organisms can be achieved even in small counts. **(Li et al., 2005)** and **(Lee et al., 2009).** Onthe other hand , PCR technique is considered as rapid technique for detection of *E.coli* as it can be detect it within 10 hours, including a 6 hours enrichment step. **(Gordillo et al., 2011).** Isolation of *E.coli* in food testing laboratories is very important, and this rapid *E.coli* detection help the strategy which will contribute to quality control in food industries. **(Takahashi et al., 2009).**

Photo (1) pointed out that agarose gel showing 5 positive strains of isolated *E. coli* by PCR. The bands of PCR products were detected which indicate presence of *E.coli* in the examined meat product samples.

Shiga-toxin producing *E.coli* :

Shiga toxin-producing *E. coli* (STEC) is a serious public health concern worldwide as such pathogen may cause diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome among food consumers. Shiga toxin produced by STEC has been considered a prime virulence factor and classified into two groups, *Stx1* and *Stx2*, on the basis of immunological properties. Though O157:H7 is the most predominant serovar isolated from sporadic cases and outbreaks , more than 100 serovars of non-O157 STEC have been isolated from animals and humans **(Abd –EL-All , 2005)**. Since most of the food poisoning cases due to STEC are related to the consumption of beef or beef products. Cattle have been considered a major reservoir of STEC. However, other vehicles, such as contaminated water, vegetables, and fruits, have been increasingly recognized as an infection source of STEC (**Shima, et al., 2006)**.

The occurrence of Shiga toxin-producing *E. coli* (STEC) in the examined meat products samples by Vero Cytotoxicity Assay (VCA) and confirmed by Multiplex Polymerase Chain Reaction and Serotyping illustrated in **table (2 , 3 and 4).**

The results recorded in Table (2) and Fig.(2) revealed that 60.8%, 50%, 43.7% , 50% and 12.5% of minced meat, beef burger, beef sausage, beef kofta and beef luncheon respectively were contaminated with shiga toxin producing *E. coli* using Vero-Cytotoxicty Assay method in relation to total number of isolated positive *E. coli* samples by conventional method.

The Cytotoxic effect of shiga toxin on vero cells was illustrated in photo(3). In the present study vero cytotoxicity assay was used as screening test for STEC. The test was done only on samples that gave characteristic colonies on sorbitol monitol agar plates, where detection of STEC was done on basis of positive VCA**.** The positive samples were confirmed by by serotyping using polyvalent and monovalent " O" *Escherchia coli* antisera, and further confirmation was done by using multiplex PCR reaction to determine the type of *Stx*.

The profound sensitivity of Vero cells to *Stx* was first observed by **Konowalchuk et al. (1977**), and cytotoxicity for this cell line remains the "gold standard" for confirmation of positive STX-producing isolates **(Byomi, 2001).**

Vero cytotoxicity Assay has played an important role in establishing a diagnosis of STEC infection, particularly where subsequent isolation of the causative organism has proven to be a difficult task. When testing such crude samples, the sensitivity is influenced by the abundance of STEC, the total amount and potency of the STX produced by the organism concerned, and the degree to which the particular STX is released from the bacterial cell , Mean while PCR technique provides a rapid and valuable diagnostic method while, detection of *Stx* by tissue culture cytotoxicity is labor-intensive, time-consuming, and cumbersome, where the results of cytotoxicity testing are generally not available for 48 to 72 hours (**Paton and Paton , 1998).**

Table (3) revealed a comparison between using Vero cytotoxicity Assay and Mutiplex PCR technique for detection of shiga-toxin producing *E. coli* in examined meat products. It is obvious that the overall sensitivity of polymerase chain reaction (PCR) = 81.57% compared with Vero Cytotoxicity Assay for detection of Shiga toxin-producing *E.coli* (*Stx1*, *Stx2* or both) in overall examined meat product samples.

However, 2 samples were VCA positive and were confirmed to be non-STEC, Since the presence of cytoxicity in a crude filtrate might be due to other bacterial

products or toxins, where positive samples should always be confirmed and typed by testing for neutralization of cytotoxicity by specific (preferably monoclonal) antibodies to *Stx1* or S*tx2* (**Smith and Scotland, 1988.).** Moreover, **Abd-El-Latif (2003)** detected two STEC strains which were positive for VCA, while only one of them was positive to PCR.

The current results agree, to some extent, with those recorded by **Hussein & Bollinger. (2005) and Hussein** (**2007**) where they that found non O157 STEC to be more prevalent in beef products than *E. coli* O157. The prevalence rates of non O157 STEC ranged from 2.4 to 30.0% in ground beef, from 17.0 to 49.2% in sausage. Testing other beef products revealed prevalence rates of 19.0% (**Zhao et al., 2001**) and 62.5% (**Samadpouet al., 1994**), respectively. **Mohammed et al (2014)** recorded that he prevalence of non-O157 STEC strains in fresh beef, ground beef and beef burger samples were 11.1% (3/27), 16.7% (5/30), and 33.3% (10/30), respectively.

Data in Table (4) illustrated that the incidence of serologically identified shiga toxin producing *E. coli* isolated from the examined minced meat samples was 11 (22%) represented as O111, O26, O103 , O119 ,O128 ,O86, O146 and O121. Accurately, such isolation of *E.coli* from the examined minced meat samples was reported by ***Fantelli and Stephan (2001) , Saleh (2001), AbdEL-Aziz (2004),***

Soliman and Tabiy (2006), Cadircl et al.,(2010) , Azab-rasha(2010) and Mohammed et al (2014) .

Moreover, the results recorded in table (4) indicated that the incidence of shiga toxin producing *E. coli* in the examined beef burger samples was 6 (12%) which were serologically identified as O111, O26, O103 ,O86, O146 and O45. Nearly similar isolation of *E. coli* was recorded by ***Saleh (2001) Abu-Hussein-Reham (2004), Soliman& Tabiy(2006) , Kassem and Sabry (2003) , Azab-Rasha(2010)*** **and** ***Mohammed et al (2014)***

Concerning the incidence of *E. coli* in the examined Beef sausage samples, five serovars were recorded and the similar one recorded as O111, O103 , O119 O86 and O45. Accurately *E. coli* serovar were previously isolated by ***Saleh (2001) , Antown & Dapgh (2009) , Badri et al.,(2009) and Azab-Rasha(2010).***

Regarding the incidence of *E. coli* in the examined beef kofta samples, 6 serovars were recorded as O111, O26 , O91 ,O128 ,O86, and O146**.** Finally, O119 was the only serovar isolated from beef luncheon. ***Azab-Rasha(2010)***

Shiga toxin producing *E. coli* (STEC) of different serovars have been isolated from human and from apparently healthy domestic animals. Many of those

isolates were typical STEC belonging to serovars O_{26}, O_{111} and O_{157} **(Karamali, 1989).** Also, verotoxin producing *E. coli* (VTEC) non O_{157} serogroups (O_{26}, O_{103}, O_{111}) are among the most important emergency food borne pathogen groups particularly O_{26} which able to cause large spectrum of illness in human as hemorrhagic colitis (HC) to hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) **(Dambrosio et al., 2007).**

Enterohaemorragic *Escherichia coli* (EHEC) constitute a subset of STEC serogroups including *E.coli* O_{157} and non - O_{157} serogroups like O_{26}, O_{111}, O_{103}, and O_{145}. STEC may be transmitted from animal reservoirs to human not only via ingestion of contaminated food or water but also by contact with STEC-positive animal or with their environment **(Alfredo et al., 2005)**.

On the other hand, EHEC shiga toxin producing *E coli* (STEC) was implicated in outbreaks of diarrhea in young children and infants. Illness caused by EHEC is typically quite severe and characterized by sudden onset of severe crampy abdominal pain followed by watery diarrhea, which later on becomes grossly bloody. Typically, there is little or no fever and the duration of illness is 2 to 9 days. Death rate in some reported outbreaks may reach 36%, while in others no death had occurred**.** Since 1982, more than 100 outbreaks of EHEC were reported in USA **(Lee et al. , 2009)**.

Enterohaemorrhagic *E.coli* (EHEC): produces two types of illness, haemorrhagic colitis and hemolytic uraemic syndrome (HUS). Haemorrhagic colitis results from colonic mucosal oedema, errosion and haemorrhage. The incubation period is 3 to 4 days. The symptoms start by sudden pain followed by watery diarrhea, nausea and vomition in the early stages of illness and abdominal distension with severe pain after the onset, disease progress over 2 days to bloody diarrhea. Haemorrhagic colitis was primarily foodbrone and was associated most frequently with *E. coli.* As recorded by **Riley(1987), Bhong et al. (2008), Lee et al. (2009) and *Xiaodong (2010).***

In the current study, *E. coli* O_{157}: H_7 failed to be detected and that may be attributed to the competency of the organisms with other microorganisms in the food or due to effectiveness of cooking and preservation. ***(Kassem and Saby, 2003)***

E. coli O_{157}: H_7 failed to be isolated from minced meat as recorded by ***Cerqueira et al. (1997), Agaogue et al. (2000)*** and ***Fantelli and Stephan (2001)*** and from burger as reported by ***Silveira et al. (1999) and Chinen et al. (2001)*** and from sausage by ***Kassem and Saby (2003) , Antown and Dapgh (2009) and Azab-rasha(2010)*** and from luncheon **(*Azab-rasha, 2010)***

Table (5) illustrates that STEC isolated from examined meat product samples prove to have virulence genes.The use of Multiplex PCR with specific primers for *Stx1, Stx2*, eae and ehly genes revealed the presence or absence of such genes in the tested isolates. The obtained results showed that *E. coli* serovars O111, O26, O103, O91, O86 and O119 proved to have *Stx1* and *Stx2* genes while, *E.coli* O128 and O121 had only *Stx1*. *E.coli* O146 had only *Stx2.*Concerning the *eae* gene responsible for the attaching and effacing lesions, *E. coli* O111 and O26 isolates prove to possess this gene .

According to, **Hornitzky et al., (2002); Jenkenis et al.,(2002) ; Bollinger (2004) and Hassanain & Ahmed (2008**) the serovars O111, O26, O103, O128, O121, O91, O86 and O119 are shiga toxin-producing *E. coli* (STEC). All of the STEC isolates produced 1, 2, 3 or 4 virulence factors (i.e. *Stx1*, *Stx2*, *Stx1&stx2* or *eae*) and were lethal to Vero (African green monkey cells). Therefore, the potential public health risk of these isolates should not be ignored.

Several researchers have been reported that *E.coli* isolates with *Stx2* gene alone predominated among cattle STEC isolates (**Sanz et al., 1998** and **Jenkins et al**., **2002).** In a few studies, most STEC being carried the *Stx2* gene in combination with *Stx1* (**Cequiera et al., 1999).** Although the prevalence of the STEC strains carrying the Stx1 alone appears to show low frequency, it is important to note

that some of non-O157 STEC serovars which commonly carry Stx1 have been associated with bloody diarrhea or hemolytic uremic syndrome in human and others have associated with diseases in cattle (**Cristancho, 2005).**

Photo (4) indicated that agarose gel shows five strains of EHEC positive for shiga toxin 1 and shiga toxin 2 and eae genes ,at size 180 bp, 255bp, 384 bp. respectively. Lane 1 and 4 include O111 and O26 which contain the three genes stx1, stx2 and eae so it contains 3 bands. Lane 2 and 3 contain PCR products of O86 and O121 it carry stx2 genes so we can detect 1 bands appear in the agarose while lane 5 include O103 , it carry stx1 and stx2 genes so we can detect 2 bands.

Photo (5) indicated that agarose gel shows six strains of EHEC positive for shiga toxin 1 and shiga toxin 2 and eae genes, at size 180 bp, 255bp, 384 bp. respectively. Lane 1 includes O111 which contain the three genes stx1, stx2 and eae so it contains 3 bands. Lane 2 and 7 contain PCR products of O146 and O86, they carry stx2 genes so we can detect one bands appears in the agarose. Lane 3 and 4 contain PCR products of O91 and O103, theycarry stx1 and stx2 genes so we can detect 2 bands.

Photo(6) pointed that agarose gel shows seven strains of EHEC positive for shiga toxin 1 and shiga toxin 2 and eae genes, at size 180 bp, 255bp, 384 bp.

respectively. Lane 1 include O111 which contain the three genes stx1,stx2 and eae so it contains 3 bands. Lane 2 and 4 contain PCR products of O128 and O45, it carry stx1 genes so we can detect 1 band appear in the agarose. Lane 3, 5 and 7 contain PCR products of O146 and O86, it carry stx2 genes so we can detect 1 bands appear in the agarose. Lane 6 contain PCR products of O119, it carry stx1 and stx2 genes so we can detect 2 bands.

In Egypt, few studies revealed the prevalence of *E.coli* O157 in meat and milk products **(Sayed et al.,2001; Mohammed, 2002,; Abd-Ell-All, 2005 and Hassan & EL-Malt, 2008)** while, many studies revealed the prevalence of non-O157 (**Byomi et al., 2001 and Abd-El-All, 2005).**

Bettleheim (2000) reported that STEC serovars other than O157:H7, such as O111, O103, O26, and O145 are emerging human pathogens predominantly in Europe, Australia, and South America.

Regarding tables(6) the results declared that 27, 36, 34, 32 and 41 samples of minced meat luncheon, beef burger, beef, sausage ,beef kofta and beef luncheon were free from *E.coli* that means the acceptability were 54%, 72%, 68% , 64% and 82% ,respectively according to the EOSQ(2005).

Regarding to these results we could conclude that minced meat and beef kofta of low acceptability this might be mainly attributed to the manner of handling each product, the number of processing operations that the product subjected to them, amount of post processing contamination and storage condition and shelf life of each product.

Biocontrol of *E.coli* O111:H4 in beef products by using of Nisin alone or combined with chemical preservatives

Nisin " bacteriocin" is an antimicrobial peptide produced by some strains of *Lactococcus lactis* **(Bender and Bender, 1995).** Nisin is used in meat technology as a chemical preservative where it has a powerful inhibitory effect against Gram-positive bacteria, but probably has not the same effect on Gram-negative ones as *E.coli* **(Delves & Gasson, 1994** and **Thomas et al., 1998)**. However, the effect of nisin when combined with various chelators such as citrate, lactate and phosphate is questionable against Gram-negative pathogens particularly *E.coli* **(Catherine & Gregory, 1995** and **Eleiwa- Nesereen, 2003).** Therefore, the for growing work is to study the action of nisin alone or through its combination with various chemical preservatives on *E.coli* in minced meat.

Tabulated results in Table (7) indicate that the addition of nisin to minced beef samples experimentally inoculated with 1×10^6/g ***E.coli*** O111:H4 by concentrations

of 10 ppm and 30 ppm could decrease their population from 1×10^6/g of $2.2\times10^4\pm9.9\times10^3$ and $5.6\times10^3 \pm 2.3\times10^3$ /g mean values after 12 hours, while the same concentrations of nisin could reduce the initial intensity of such pathogen to averages of $7.3\times10^3 \pm 2.8\times10^3$ and $2.6\times10^3\pm1.7\times10^3$ /g after 24 hours, whereas more reduction was revealed when the initial count declined to mean values of $5.2\times10^3 \pm 2.3\times10^3$and $1.4\times10^3 \pm 7.7\times10^2$ /g after 48hours, respectively. Such variations in population were significant ($P<0.05$) as a result of nisin concentrations and durations of treatment. Accordingly, the addition of nisin alone whatever its concentration was not effective for complete destruction of ***E.coli*** O111:H4 inoculated into minced beef samples. The current results agree, to some extent, with those recorded by **Demel et al.(1996), Hassan(1999)** and **Gill &Holly(2004)** who reported that all ***E.coli*** were relatively resistant to the bactericidal effect of nisin.

In general, nisin has a wide a spectrum of activity against Gram-positive bacteria including spore-formers but its action is not significant against Gram-negative bacteria **(Thomas et al.,1998)**. In this respect, Gram-positive bacteria are characterized by presence of high contents of anionic lipids in their cell membranes which can be easily penetrated by nisin **(Masschalck et al., 2003)**. Thus, disruption of the bacterial cell membrane results in the passage of nisin to

the cytoplasmic membrane as the site of nisin action **(Carneiro et al., 1998)**. On the other hand, the resistance of *E.coli* as Gram-negative bacteria against nisin depends on the type of lipopolysaccharid of the cell membrane. Consequently, addition of an agent to change the nature of the outer membrane of *E.coli* is very necessary to render the organism sensitive to nisin **(Ganzle et al., 1999).**

Table (8) reveals that the combination of nisin (10 ppm) and sodium acetate (0.2%) has a slight effect on the viable count of *E.coli* inoculated into minced beef samples (1×10^6/g) to become of mean values of $1.4\times10^4 \pm 7.9\times10^3$, $5.0\times10^3 \pm 2.5\times10^3$ and $2.1\times10^3 \pm 8.6\times10^2$ organisms per gram after 12, 24 and 48 hours from the incorporation of such formula in tested samples, respectively. Furthermore, nisin at concentrations of 30 ppm mixed 0.2% sodium acetate decreased the initial intensity of inoculated *E.coli* (1×10^6/g) to average of $2.9\times10^3 \pm 1.3\times10^3$, $1.3\times10^2 \pm 9.3\times10^2$ and $4.4\times10^2 \pm 2.9\times10^2$ organisms per gram after 12, 24 and 48 hours from the incorporation of such formula in tested samples, respectively. Although the concentrations of nisin and durations of treatment had significant effect ($p < 0.05$) on viability of *E.coli*, yet the complete destruction of this organism was not attained by nisin and sodium acetate mixture. Similar findings were obtained by **Stevens et al. (1997),** who failed to destroy *E.coli* organisms by nisin and sodium acetate mixture.

Data in table (9) declare that the combination of nisin (10 ppm) and potassium sorbate (0.2%) has a slight effect on the viable count of *E.coli* inoculated into minced beef samples (1×10^6/g) to become of mean values of $1.3\times10^4 \pm 95\times10^3$, $6.1\times10^2 \pm 2.5\times10^2$ and $5.4\times10^2 \pm 2.0\times10^2$ /g after 12, 24 and 48 hours from the incorporation of such formula in tested samples, respectively. Furthermore, nisin at concentrations of 30 ppm mixed 0.2% potassium sorbate decreased the initial intensity of inoculated *E.coli* (1×10^6/g) to average of $2.3\times10^3 \pm 9.7\times10^2$, $3.2\times10^2 \pm 2.4\times10^2$ and $6.6\times10 \pm 2.4\times10$ organisms per gram after 12, 24 and 48 hours from the incorporation of such formula in tested samples, respectively. Although the concentrations of nisin and durations of treatment had significant effect ($p < 0.05$) on viability of *E.coli*, yet the complete destruction of this organism was not attained by nisin and potassium sorbate mixture. Similar findings were obtained by **Hassan(1999)** and **Eleiwa- Nesereen (2003)** who failed to destroy *E.coli* organisms by nisin and potassium sorbate mixture.

Actually, potassium sorbate inhibits certain amino acids uptake by *E.coli* interfering with its growth. However, the action of potassium sorbate is greatly depentant on PH of the food article where the low PH (1-4) had an apparent synergistic inhibition action caused by sorbate on the amino acid uptake by *E.coli* **(Eklund, 2003).**Therefore, the minor role of potassium sorbate with nisin for

destruction *E.coli* in the present study may be attributed to the fact that the PH of fresh minced meat is above 5.5 at which the action of potassium sorbate is significantly affected. **Eleiwa- Nesereen (2003)**

Table (10) decleared that nisin (10 ppm and 30 ppm) combined with sodium lactate (0.2 %) could decrease the initial count of *E.coli* in tested minced beef samples from 1×10^{6}/g to mean values of $4.0\times10^{2} \pm 2.0\times10^{2}$ and $15\times10 \pm 7.5\times10$ /g after 12 hours from adding this preservative formula in the tested samples, respectively. While, the incorporation of nisin either 10 or 30 ppm with 0. 2% Sodium lactate lead to a complete destruction of *E.coli* after 24 and 48 hours of this treatment.

It is postulated that Sodium lactate destabilize the cell membrane of *E.coli* and other Gram-negative bacteria by chelating Mg++ and /Ca++ that affecting its permeability to be sensitive to the action of nisin **(Catherine & Gregory, 1995** and **Tipayanate et al., 1999).** It is concluded that addition of nisin in combination with sodium lactate is effective for destruction of *E.coli.* Consequently, from the economic point of view, the later formula is wonderful for the meat processors to produce a product of good keeping quality meat by adding the correct preservative against the target organism instead of application of many other preservative of higher cost and lower effect.

Finally, the present results allow to conclude that the best formula to destroy of *E.coli* O111H4 in minced beef is the application of nisin by concentration of 10 ppm and 30 ppm coupled with sodium lactate (0.2%) for 24 and 48 hours to obtain a maximum margin of human safety against such serious pathogen.

6- CONCLUSION AND RECOMMENDATIONS

In the present study it is obviously that some of meat products were contaminated with *E.coli* especially *shiga- toxin producing E.coli* specially minced meat, beef kofta and beef sausage, this may be attributed to contamination during further processing stages or using of contaminated materials as spices or contaminations during handling and from surrounding environment therefore we are in need to use modern and rapid technique for detection of microorganisms as PCR which is accurate and time saving technique. And also concluded that the examined meat products consumed in Egypt are considerably contaminated with a variety of non-O157 STEC serovars, and hence consumption of such products may constitute a potential health risk for consumers and that most of isolated strains carry virulence genes, especially Shiga toxins , which are implicated in human diseases worldwide. In addition, nisin and sodium lactate could be used in meat products to destroy *E.coli*.

Therefore the following recommendations should be carried out:-

6.1 practice in the laboratory

6.6.1- Polymerase chain reaction (PCR) should be used in diagnosis and detection of food borne pathogen as it is quick and accurate method and saves time and labor.

6.6.2-Using nisin and sodium lactate in meat products to destroy *E.coli* is important in addition of addative.

6.2. Quality choice :

6.2.1. A good quality raw material should be used in the manufacture of meat products, because the finished product is substantially influenced by the characteristics of the fresh raw materials.

6.1.2. Raw materials should be inspected on delivery by properly trained staff.

6.2.3. Raw materials should be stored under proper conditions and each batch should be identified.

6.2.4. High quality spices and food additives must be free from any contaminants.

6.3. Hygienic practices on meat processing plant :

6.3.1. The cleanliness of premises, stalls, vehicles and equipments used for manufacture of meat products should be carried out daily .

6.3.2. meat handlers should be healthy and have medical certificate to avoid cross contamination.

6.3.3. Practices should be improved by maintaining clean water supply, disinfecting the environment periodically and sanitary disposal of wastes combine with well- constructed enclosure.

6.3.4. Laboratory control for the various steps of production should be conducted to detect probable sources of contamination.

6.3.5. Activities should be evaluated for situations in which the workers may contaminate the product. Moreover, entry to production halls should be restricted to personnel necessary to carry out operations efficiently.

6.3.6. Each process or step should also be evaluated for its effect on increasing or decreasing the number of bacteria.

6.3.7. Processes, which increase the product contamination, should be eliminated or modified. Alternatively, a subsequent process can be added to modify, reduce or eliminate the problem.

6.3.8. The possibility of the product contamination particularly by contact with raw materials or by hands or workers should be avoided.

6.3.9 Generally, application and implementation of Hazard Analysis Critical Control Point (HACCP) system as a hazards control system should be done in meat processing group to decide on whether good manufacturing practice (GMP) is being done and to ensure a maximum safety to consumers.

6.3.10. Specialists should be not only to detect the errors and defects but also to ensure that errors are corrected and defects are not repeated and should enforce adequate control measure through periodical inspection.

6.3.11. Periodical sanitation of processing group by adequate sanitizers. Approximately, 250-1000 ml of sanitizer should be applied for one square meter.

6.4.Hygienic practices on markets :

6.4.1. The temperature of freezing storage cabinets should be checked periodically at-18ºC.

6.4.2. The freezing age of the meat product must be considered for the shelf life of that product.

6.4.3. Distributor must be informed not to put the power “off” over night.

6.4.4. Storage rooms should have physical barriers for rodents and insects.

6.4.5. Personnel involved in the food trade must be subjected to health inspection and supervision periodically.

6.5. Hygienic practices during preparation of meat products :

6.5.1. Thawing of frozen raw meat in refrigerator is recommended at temperature 5ºC.

6.5.2. Meat products after cooling should not be held at room temperature

6.5.3. Meat products must be through cooked to permit the internal temperature to be lethal to vegetative bacteria (80ºC for 3 minutes).

6.6. Education of meat handlers and consumers :

Education and training of the meat handlers are the key stones of effective quality control

6.6.1. Managers, veterinarians and supervisors in the processing group should train the workers under their direction for safe production of meat products and proper sanitation of equipments

6.6.2. Workers must be learned the basic principles of meat borne diseases and their control.

6.6.3. Consumers should understand the importance of adequate refrigeration of raw meat products, hand washing before preparation and proper cooking of such meat products.

6.6.4. Product label can help and giving advice to the consumer wherever possible about handling and storage of the product.

7-Summary

A total of 250 random meat product samples (50 each of minced meat, beef burger, beef sausage, beef kofta and beef luncheon) were collected from different super markets at different localities for detection of *Escherichia coli* using conventional method and Polymerase chain reaction. Conventional method indicated that *E. coli* could be isolated from of minced meat, beef burger, beef sausage, beef kofta and beef luncheon in a percentage of 46%, 28%, 32% , 36% and 16% respectively. While Polymerase chain reaction detected *E. coli* in of minced meat, beef burger, beef sausage, beef kofta and beef luncheon in a percentage of 38%, 22%, 30%, 32% and 12% respectively.

The present study was planned to investigate the presence of Shiga toxin-producing *E. coli* (STEC) in meat products sample as examined by Vero Cytotoxicity Assay (VCA) and confirmed by Serotyping and Multiplex Polymerase Chain Reaction.

It is obvious that in the Vero cytotoxicity assay the percentage of shiga-toxin producing *E.coli* were 60.8%, 50%, 43.7% , 50% and 12.5% of minced meat, beef burger, beef sausage, beef kofta and beef luncheon, respectively in relation to total number of isolated positive *E. coli* samples.while by Mutiplex PCR method the percentage of shiga-toxin producing *E.coli* were 47.8%, 42.8%, 31.3%, 44.4% and 12.5% of minced meat, beef burger, beef sausage, beef kofta and beef luncheon, respectively

The serological identification of shiga-toxin producing *E.coli* isolated from the examined minced meat samples were O111, O26, O103 , O119 ,O128 ,O86, O146 and O121. While for beef burger samples, the isolated serovars were O111, O26 , O103 ,O86, O146 and O45. But the isolated serovars from beef sausage were O111, O103 , O119 O86 and O45.The isolated serovars from beef kofta were O111, O26 , O91 ,O128 ,O86, and O146. Finally the only one serovars isolated from beef luncheon was O119.

The use of Multiplex PCR with specific primers for *Stx1, Stx2 ,eae* and ehly genes revealed the presence or absence of these genes in the tested isolates. The obtained results showed that the isolates *E. coli* O111, O26, O103, O91, O86 and O119 had *Stx1* and *Stx2* genes while, *E.coli* O128 and O121 had only *Stx1*. *E.coli* O146 had only *Stx2.*Concerning the *eae* gene responsible for the attaching and effacing lesions, *E. coli* O111 and O26 isolates possessed this gene .

The second part of this study was planned to explore the effect of Nisin alone or combined with chemical preservatives on the growth of *E.coli* O111:H4. The obtained results allow to conclude that the best formula to destroy all organisms of *E.coli* including O111: H4 in minced beef is the application of nisin by concentration of 10 ppm and 30 ppm coupled with sodium lactate(0.2%) for 24 and 48 hours to obtain a maximum margin of human safety against such serious pathogen.

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الملخص العربى

تعتبر اللحوم من أهم الاغذية التى يحتاج الانسان اليها نظرا لاحتوائها على العديد من العناصر الغذائية اللازمة للجسم .

وتعتبر منتجات اللحوم من أهم الاغذية التى يقبل عليها عدد كبير من المستهلكين فى العالم وذلك لطعمها الشهى وسهولة اعدادها بالاضافة الى انخفاض ثمنها ولانها تحتوى على نسبة عالية من البروتين الحيوانى .

ونظرا لتعرض منتجات اللحوم للتلوث بالعديد من الميكروبات الممرضة من مصادر مختلفة ابتداء من عملية التصنيع والاعداد والتجهيز للمنتج حتى وصولها الى المستهلك مما يؤدى الى سرعة فسادها وتلفها نتيجة نمو فقد اجريت هذه الدراسة علي 250 عينه من منتجات الميكروبات ممسببة خطورة صحية على المستهلك اللحوم (اللحم المفروم والنقانق والبرجر والكفتة البقرى واللانشون البقري) بواقع 50 عينه من كل منتج والمجمعه من مصادرمختلفة وذلك للكشف عن وجود ميكروب الاشيرشيا كولاي و ميكروب الاشيرشيا

كولاي المفرز لسموم الشيجا توكسين في منتجات اللحوم بالطرق التقليدية وبالطرق الحديثة مثل تقنية PCR تفاعلات البلمرة المتسلسله و ايضا دراسة مدى تواجد بعض الجينات المميزة لعترات الاشريشيا كولاى المسببة للنزيف والسموم المفرز لها والناتجة عن تواجده ونموه والكشف عنها باستخدام البيولوجيا الجزيئية.

- تم الكشف عن وجود ميكروب الاشيرشيا كولاي باستخدام طرق العزل التقليدية النقانق بنسبة 32%وفى الكفتة البقرى بنسبة 36% وفي اللانشون بنسبة 16%.
- باستخدام تفاعل البلمره المتسلسل للكشف على نفس الميكروب فقد ثبتا وجوده في كل من اللحم المفروم بنسبة 38% وفي البرجر البقري بنسبة 22% وفي النقانق بنسبة 30%وفى الكفتة البقرى بنسبة 32% وفي اللانشون بنسبة 12%.

- تم فحص هذه العينات للبحث عن ميكروب الاشريشيا كولاى المفرز لتوكسين شيجا اولا بطريقة استخدام ("VCA" vero cell assay) علي العينات الايجابية. بعد ذلك تم تصنيف العينات الايجابية لاختبار VCA سيرولوجيا وكذلك تم تاكيدها باستخدام تفاعل البلمرة المتسلسل المتعدد(Multiplex PCR) لتحديد نوع الجين المسئول عن افراز سموم شيجا.

- تم الكشف عن وجود ميكروب الاشيرشيا كولاي المفرزة لسموم الشيجا باستخدام vero cell assay "VCA فوجد في كل من اللحم المفروم بنسبة 60.8% وفي البرجر البقري بنسبة 50% وفي النقانق بنسبة 43.7%وفى الكفتة البقرى بنسبة 50% وفي اللانشون بنسبة 12.5% بالنسبة للعينات الايجابية لميكروب الاشيرشيا كولاي .
- باستخدام تفاعل البلمره المتسلسل المتعدد للكشف عن ميكروب الاشيرشيا كولاي المفرزة لسموم الشيجا فوجد في كل من اللحم المفروم بنسبة 78.57% وفي البرجر البقري بنسبة 85.71% وفي النقانق بنسبة 71.42%وفى الكفتة البقرى بنسبة 88.88% وفي اللانشون بنسبة 100% بالنسبة للعينات الايجابية لميكروب الاشيرشيا كولاي المفرزة لسموم الشيجا باستخدام vero cell assay "VCA .
- تم تصنيف المعزولات لميكروب الاشيرشيا كولاي المفرزة لسموم الشيجا فوجد في كل من اللحم المفروم O111, O111, O26 , O103 وفي البرجر O26, O103 , O119 ,O128 ,O86, O146وO121 O86, O146,و O45 وفي النقانق O86 O119 , O103 , O111, و O45 وفى الكفتة البقرى ,O86, O128 ,O91 , O26 ,O111وO146 وفي اللانشونO119 .

- استخدام تفاعل البلمرة المتسلسل المتعدد(Multiplex PCR) بواسطة بادئات لجينات شيجا توكسين1 *Stx1* وشيجا توكسين 2 *Stx2* و *eae* و *Hyl* عن وجود او غياب هذه الجينات في العينات وكانت النتائج كالتالي: وجود شيجا توكسين 1 و شيجا توكسين 2 معاً في معزولات O111, O26, O103, O91, O86 وO119. وجود شيجا توكسين 1 فقط في معزولات

O128وO121 . وجود شيجا توكسين 2 فقط في معزولات O146 . وجود جين *eae* في معزولات O111 وO26 .

- أثبتت الدراسه ان تفاعل البلمرة المتسلسل دقيق وسريع في الكشف عن الميكروبات الممرضة وسمومها كما انها توفر الوقت والعمالة داخل المعمل
- تناولت الدراسة تأثير كل من nisin , sodium acetate ,potassium sorbate and sodium lactateو مدة تخزين اللحوم ومنتجاتها على كثافة نمو ميكروب الاشيرشيا كولاي .
- أثبتت الدراسه ان nisin and sodium lactate له تأثير فعال ضد نمو ميكروب الاشيرشيا كولايO111 H4 .

وقد تم دراسة ومناقشة الاهمية الصحية للميكروبات المعزولة ومصادر تلوث منتجات اللحوم تحت الفحص بالاضافة الى وضع التوصيات اللازمة لتحسين جودة هذه المنتجات .

جامعة مدينه السادات

كلية الطب البيطري

قسم الرقابة الصحية على الأغذية

(الرقابة الصحية على اللحوم ومنتجاتها)

تقرير لجنة الحكم والمناقشة

قامت لجنة الحكم والمناقشة بجلستها المنعقدة في صباح يوم السبت الموافق 10/ 1/ 2015 الاشريشيا كولاى بفحص الرسالة وعنوانها " مدى تواجد والتحكم الحيوى لميكروب المفرزة لسموم الشيجا فى بعض منتجات اللحوم البقرى " ووجدت أنها قيمة إذ اشتملت على بحوث هادفة تناولت مواضيع لها أهميتها من الناحية العلمية والصحية والتطبيقية .

لذلك

توصى اللجنة بترشيح السيد/ ط . ب. رياض ربيع مصطفى احمد شاويش للحصول على درجة الدكتوراه في العلوم الطبية البيطرية تخصص الرقابة الصحية على اللحوم ومنتجاتها.

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قسم الرقابة الصحية على الاغذية

والتحكم الحيوى لميكروب الاشريشيا كولاى مدى تواجد المفرزة لسموم الشيجا فى بعض منتجات اللحوم البقرى

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(2015)