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Prevalence and Molecular Characterizations of *Escherichia coli* in Meat Products

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ABSTRACT

Meat products is considered a rich source of proteins, essential amino acids, B complex vitamins and minerals. So, it considers a highly favorable environment for the pathogenic bacteria growth. A total of 200 random samples of meat products (minced meat, kofta, beef burger, luncheon and sausage) ,40 of each, collected from small scale shops and markets at Menoufia Governorate, Egypt. The collected samples were examined for isolation, serological identification and molecular characterization of *E. coli* by using PCR technique. The results showed that the isolated *E. coli* from the examined samples by the percentage of 20% (8/40) ,25% (10/40) ,42.5% (17/40) ,50% (20/40) ,37.5% (15/40), respectively. The isolated *E. coli* was serologically identified as O55:H7, O78, O119:H6, O124, O127:H6 and O146:H21. PCR results showed that shiga toxin 2 gene (*stx2*) was detected in O78 and O146:H21 while, shiga toxin 1 gene(*stx1*) and shiga toxin 2 gene(*stx2*) were detected in O55:H7 ,O119:H6 and O127:H6, also, *E.coli* O127:H6 strain was positive for intimin gene (*eaeA*).

Keyword: Meat products, *E. coli*, Shiga toxins.

INTRODUCTION

Meat and meat products have not only been following convenience trends of food ,but also they have been at the heart of them (Leroy and Degreef , 2015).Meat products such as minced meat ,beef burger ,kofta, sausage and luncheon are highly demanded and considered more attractive for consumers than fresh meat due to their high nutritive value, reasonable price, good taste, quick easily prepared and also easily serving. Although the importance of meat products for consumers, yet they can be contaminated with several types of food borne pathogens from different sources during handling, preparation, and storage practices (Taulo *et al.*, 2008). *Escherichia coli* is a non-sporulating, Gram negative facultative anaerobe, which has in large-intestinal more than 500 species content in animals and reptiles. *E. coli*

strains are categorized into six pathotypes: enterohaemorrhagic *E.coli* (*EHEC*), enteropathogenic *E.coli* (*EPEC*), enterotoxigenic *E.coli* (*ETEC*), enteroaggregative *E.coli* (*EAEC*), entero-invasive *E.coli* (*EIEC*) and diffusely adherent *E. coli* (*DAEC*).The most common syndromes can be caused from one of these pathotypes include gastrointestinal diseases, urinary tract infections (UTIs) and sepsis/meningitis. Pathogenicity mechanism of *E. coli* consists of adherence to specific receptors on the intestinal epithelial cells, colonization of mucosal site, evasion of host defenses, multiplication, and damage to host cells (Aminzare *et al.*, 2017). Shiga toxin-producing *Escherichia coli* (*STEC*) strains, including those of O157:H7 and the “big six” serogroups (O26, O45, O103, O111, O121, and O145) are food-borne pathogens which cause a serious health threat to Humans (Jiang *et al.*,

2015). The commensal *E. coli* strains from the normal intestinal flora are harmless to the host and only cause disease when the gastrointestinal barriers are breached or in immune compromised hosts. Although, some specific *E. coli* strains represent primary pathogens with an 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. Different pathotypes caused by specific combinations of virulence factors based on the various human diseases caused by *E. coli* (Li *et al.*, 2005).

The aim of the present Article is to study prevalence and molecular characterization of isolated *E. coli* from examined samples of beef burger, minced meat, kofta, sausage and luncheon.

MATERIAL AND METHOD

Collection of samples: A total of 200 samples collected randomly from raw products of meat; minced meat, beef burger, kofta, sausage and luncheon ,40 from each within 250 g, were collected from small scale shops and markets with different sanitation levels at Menoufia Governorate, Egypt. The collected samples were transferred directly to the laboratory of Food Hygiene of Animal Health Research Institute, Shebin El-Koom Branch .in an ice box under complete aseptic conditions without undue delay, thawed at room-temperature to be examined bacteriologically for isolations and identifications of *E. coli* strains.

Conventional identification of E. coli

Preparation of samples: we prepared the samples depending on the technique recommended by APHA (1992) as follows: 25 grams from each beef meat product samples were carried in sterile sac, then added to it 225 ml from 0.1 % sterile peptone water under aseptic conditions. then homogenized every sample in a

blender was adjusted at 2000 r.p.m for 1-2 minutes to obtain a mix of them.

Isolation and identification of Escherichia coli: (APHA, 1992).

Enrichment from the original dilution (25g/225ml), take 1ml and put into the tube of MacConkey broth with inverted Durham's tubes. Take the control tubes and inoculated one for incubation 24 hours at 37°C. positive tube for suspected *E. coli* having acid and gas production, were considered positive for suspected and the results were recorded. One ml from each positive tube was added into another MacConkey broth tube for incubation 48 hours at 44.5°C.

Plating media: MacConkey agar medium was streaked separately by loop from positive MacConkey broth tubes and incubated at 37°C for 24 hours. Suspected lactose fermented colonies were picked up and plating onto Eosin Methylene Blue Agar medium (EMB), then incubated 24 hours at 37°C. metallic green colonies were suspected of *E. coli*. For further identification suspected colonies were picked up and put into nutrient agar slope tubes.

Conventional Identification Of Suspected E.Coli : Was Carried Out According To (Koneman *et al.*, 1997).

Serological Identification: The isolates were identified serologically according to (Kok *et al.*, 1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

Molecular identification of isolated E. coli was done according to (Sambrook *et al.*, 1989)

Primer sequences of E. coli used for PCR identification system **Materials used for PCR:**

Application of PCR for identification of shiga toxins (*stx1* & *stx2*) and intimin (*eaeA*) genes of *E. coli* was performed basically by using primers (Pharmacia Biotech) as shown in the following (table A):

Table (1): Primer sequences for PCR identification

Primer	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
<i>stx1</i> (F)	5' ATAAATCGCCATTCGTTGACTAC '3	180	Paton and Paton (1998)
<i>Stx1</i> (R)	5' AGAACGCCCACTGAGATCATC '3		
<i>Stx2</i> (F)	5' GGCAGTGTCTGAAACTGCTCC '3	255	
<i>Stx2</i> (R)	5' TCGCCAGTTATCTGACATTCTG '3		
<i>eaeA</i> (F)	5' GACCCGGCACAAGCATAAGC '3	384	
<i>eaeA</i> (R)	5' CCACCTGCAGCAACAAGAGG '3		

RESULTS and DISCUSSION

Results given in table (2) revealed that the incidence of *E.coli* in the examined samples of meat products (minced meat , beef burger, kofta , sausage and luncheon) were 20% (8/40) ,25% (10/40) ,42.5% (17/40), 50% (20/40) ,37.5% (15/40) , respectively .

Table (2): Incidence of Enteropathogenic *E. coli* strains in the examined meat products (n=40 of each) samples

Meat products	No.+ve sample	%
Minced meat	8	20
Beef burger	10	25
Kofta	17	42.5
Sausage	20	50
Luncheon	15	37.5
Total (200)	70	35

Table (3): Incidence of *E. coli* serovars in the examined samples of meat products.

<i>E.coli</i> serovars	Meat products		Minced meat		Beef burger		Kofta		Sausage		Luncheon		Strain characteristics
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
O55: H7	2	5	3	7.5	1	2.5	2	5	-	-	-	-	EHEC
O78	-	-	1	2.5	3	7.5	6	15	3	7.5	3	7.5	EPEC
O119: H6	4	10	-	-	5	12.5	1	2.5	2	5	2	5	EPEC
O124	1	2.5	-	-	3	7.5	5	12.5	1	2.5	1	2.5	EIEC
O127: H6	-	-	1	2.5	5	12.5	2	5	3	7.5	3	7.5	ETEC
O146: H21	1	2.5	5	12.5	-	-	4	10	6	15	6	15	EPEC
Total	8	20	10	25	17	42.5	20	50	15	37.5	15	37.5	

EPEC = Enteropathogenic *E. coli*

ETEC = Enterotoxigenic *E. coli*

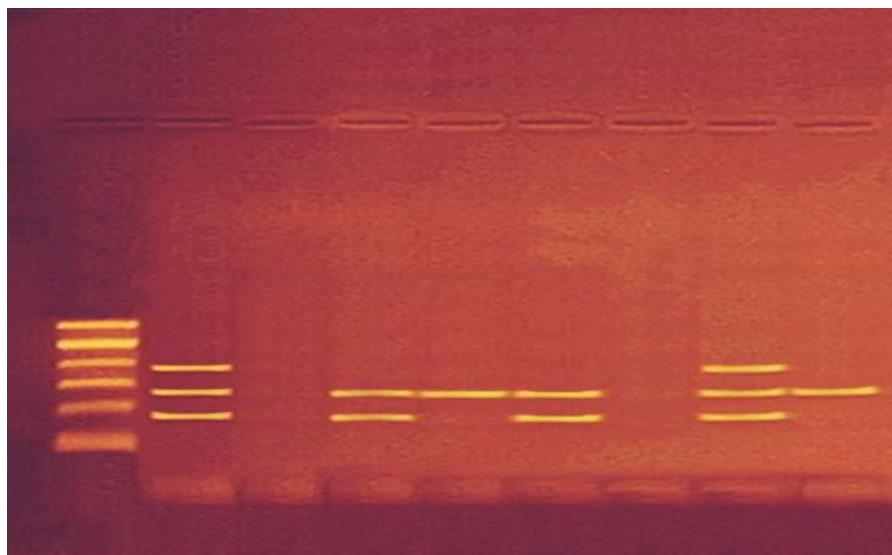
EIEC = Enteroinvasive *E. coli*

EHEC = Enterohaemorrhagic *E. coli*

Table (4): Acceptability of the examined samples of meat products based on their contamination with *E. coli* with "EOS" (2005) (n=40 of each).

Meat products	Accepted samples		Unaccepted samples	
	No.	%	No.	%
Minced meat	32	80	8	20
Beef burger	30	75	10	25
Kofta	23	57.5	17	42.5
Sausage	20	50	20	50
Luncheon	25	63.5	15	37.5
Total (200)	130	65	70	35

* Egyptian Organization for Standardization "EOS" (2005). Where ES stipulated that meat products should be free from *E. coli*.

**Fig. (1):** Agarose gel electrophoresis of multiplex PCR of *stx1* (180bp), *stx2* (255 bp) and *eaeA* (384 bp) virulence genes for characterization of *E. coli*.

Lane M: 100 bp ladder as molecular size DNA marker. **Lane C+:** Control positive *E. coli* for *stx1*, *stx2* and *eaeA* genes.

Lane C-: Control negative. **Lanes 1 (O55) & 3 (O119):** Positive *E. coli* for *stx1* and *stx2* genes.

Lanes 2 (O78) & 6 (O146): Positive *E. coli* for *stx2* gene. **Lane 4 (O124):** Negative *E. coli* for *stx1*, *stx2* and *eaeA* genes.

Lane 5 (O127): Positive *E. coli* for *stx1*, *stx2* and *eaeA* genes.

Table (5): Incidence of virulence genes of pathogenic *E. coli* isolated from the examined samples of meat products (n= 6 strains).

<i>E. coli</i> Serovars	<i>stx1</i>	<i>stx2</i>	<i>eaeA</i>
O55: H7	+	+	-
O78	-	+	-
O119: H6	+	+	-
O124	-	-	-
O127: H6	+	+	+
O146: H21	-	+	-

Incidence of *E. coli* in the examined minced meat samples were 20% (8/40). Comparatively lower rates were reported by (Jehan *et al.*, 2016) "(18%)", Fantelli and Stephan (2001) "(2.3%)" and (Emara *et al.*, 2016) "(4%)." Relatively higher results were reported by (Mousa *et al.*, 2011) "(18=36%)", (Shawish *et al.*, 2014) "(38%)" and (Tarabees *et al.*, 2015) "(42.5%)." clear differences in the contamination rates of

bacteria were noted where raw meats are often contaminated with food-borne microorganism; also, there are marked differences in the incidence of that microorganism in different meats. Raw meats are a good vehicle for transmitting food-borne diseases, so we must increase implementation of hazard analysis of critical control point (HACCP) and education of

consumer to food safety efforts (Zhao *et al.*, 2001).

Incidence of *E. coli* in the examined Beef burger was 25% (10/40). Comparatively higher results were reported by (Mousa *et al.*, 2011), (Osman *et al.*, 2018) and (Hassanien *et al.*, 2016) 32%, 30% and 28%, respectively. While, the results were nearly agreed with (Shawish *et al.*, 2014) “22%”, Relatively lower results were reported by (Saleh *et al.*, 2010) and (Shaltot *et al.*, 2015); 12%, 10%, respectively. *E. coli* is usually used as surrogate indicator, its presence in food generally shows direct and indirect fecal contamination (Clarence *et al.*, 2009). The possible sources of pathogens contaminated ready to eat meat products were inadequate sanitary practices or insufficient heat treatment with presence of pathogens on different surfaces occasionally contaminated the final product. (El-Dosoky *et al.*, 2013).

Incidence of *E. coli* in the examined Kofta was 42.5% (17/40). The higher rate of microbial contamination of the examined samples of kofta reported by (Hassan *et al.*, 2018) (46.67%). Lower incidence reported by (Hassanien, 2004) “8%” (Al-Mutairi, 2011) “28%” “The obtained results nearly agree with (Hassanin *et al.*, 2014) “40%”. Meat products were contaminated with *E. coli* especially (kofta). The high rate of contamination of these meat products due to the unhygienic and poor sanitary conditions during the handling and processing, which can be reduced by application of good manufacture practices (Shaltout, 2019).

Incidence of *E. coli* in the examined sausage was 50% (20/40). Comparatively lower results were reported by Osman *et al.* (2018) “(30%), (Shaltot *et al.*, 2015) “25%” and (Abd El Tawab *et al.*, 2015) “22,6%”. But (Al-Mutairi, 2011) failed to isolate *E. coli* from sausage. Relatively higher results reported by (Tarabees *et al.*, 2015) “57,5%”. The variations of results between different author because the residual microflora contaminating the surfaces and the equipment such as the machines, the tables and the knives during sausage manufacture. The variability of the residual contamination emphasized the different cleaning, disinfecting, effectiveness of hygienic measure and manufacturing practices routinely followed by

these small-scale processing units. (Talon *et al.*, 2007). Contaminated beef was suspected to be the source of infection due to lack of heat-treatment, delayed start of fermentation and a short curing period in cold temperature were identified as the main factors enabling the bacteria survival. If curing conditions are inadequate, *E. coli* can survive throughout the entire production process of fermented sausage (Sartz *et al.*, 2008). The examined sausage samples are more contaminated than other samples and these due to high fat percentage and large casing diameter of sausage which enhance the growth of *E. coli*, also recipe type used in sausage, improper processing and improper storage (Heir, *et al.*, 2010).

Incidence of *E. coli* in the examined luncheon was 37,5% (15/40) comparatively lower results were obtained by (Hassanien, 2004), (Mousa *et al.*, 2011), (Shawish *et al.*, 2014) and (Tarabees *et al.*, 2015); 4%, 6%, 12% and 20% respectively. The presence of *E. coli* in the examined samples indicated for fecal contamination due to improper handling and unhygienic conditions. (Hashim, 2003). The hazard that contaminated food poses to customers will hang on type, extent of contamination and the potential of the food-stuff to foster growth of contaminant bacteria and the kind of preparation prior to consumption. In the case of the luncheon meat, the consumption will be in salads, sandwiches and other dishes that will not undergo a cooking step. Thus, the contamination occurs during the slicing and packaging of luncheon meat at supermarkets may represent an additional concern to the food safety (Mottin *et al.*, 2011).

The given results in table (3) revealed that the isolated serovars of *E. coli* in minced meat were serologically identified as O55:H7, O119:H6, O124 and O146:H21. These results agree to some extent with (Al-Mutairi, 2011) who isolated (O55, O119, O146) and (Hassanin *et al.*, 2014) who isolated O119: H6 and O124. On the other side, these results disagree with (Shaltout, 2017) who Cannot isolate any similar serovars.

The given results in table (3) revealed that the isolated serovars of *E. coli* from Beef burger were serologically identified as O55:H7, O78, O127:H6 and O146:H21. These result nearly agree with (Abd El Tawab *et al.*, 2015) who

isolated(O55:H7andO78),Al-Mutairi(2011)who isolated(O78, O55 and O146) and with (Hassanin *et al.*, 2014)who isolated (O127:H6). on the other hand, these results disagree with (Klein *et al.*,2002) who isolated O157:H7 and (Essa *et al.*, 2009) isolated 5 strains of *E. coli* O₁₁₁K₅₈.O103:H2 and O118:H16.

The results recorded in table (3) revealed that the isolated serovars of *E.coli* in kofta were serologically identified as O55:H7,O78 , O119:H6 , O124 and O127 :H6 .These results agree with (Hassan *et al.*,2015) Who isolate O55 : H7 ,O119 : H4 , O124 , O127 : H6 and O 128 : H2 with various percentages and with (Emara *et al.*,2016) who isolated (O119: H4, O127: H6, O55: H7, O114: H21 and O124) .and disagree with (Selim *et al.*,2013) who isolated serotype O125, O158.

The results given in table (3) revealed that the isolated serovars of *E.coli* in sausage were serologically identified as O55:H7 ,O78, O119:H6 ,O124 ,O127:H6 and O146:H21 .these results disagree with(Hassanien ,2004)Who isolated O55 : K59 (B5) O111 : K58 (B14), O124 : K72 (B17) and O124 : K67 (B12) and these results agree with (Al-Mutairi ,2011) who isolated (O78, O126, O55, O119, O146 and O126) .

The results obtained in table (3) revealed that the isolated serotypes of *E. coli* in luncheon were serologically identified as O78, O119:H6, O124, O127:H6 and O146:H21. these results disagree with Essa *et al.*, (2009) who isolated two strains *E. coli* O₁₂₈K₆₇ and only one strain *E. coli* O₁₂₆K₇ and Awadallah *et al.*, (2014) who isolated coli O55:K59, O26:K60, O111:K58, O124:K72 and O128:K67 And agree with Hassan *et al.*, (2018) who isolated , O124 ,O119:H6 and O127:H6. Every treatment done to the meat from the point of slaughtering until it is ready for consumption will add to the bacterial load of this meat. Thus, meat products are considered as a major vehicle of most reported food borne outbreak and may be contaminated with several types of organisms through long chain of preparation, handling of raw meat, equipment, processing, distribution storage and retailing. (Shawish *et al.*, 2014).

The results given in table (4) revealed that the accepted samples of *E. coli* in minced meat “32”,

beef burger” 30”, kofta (23), sausage (20) and luncheon (25). With the percentage of (80%), (75%), (57.5%), (50%) and (63,5%) respectively. while unaccepted samples of *E.coli* in minced meat”8”, beef burger”10” , in kofta “17” , in sausage”20” and in luncheon “15” with the percentage of (20%), (25%) , (42.5%) , (50%) and (37.5%) respectively. According to the criteria of “EOS” No1694-2005 for minced meat, the criteria of “EOS” No1688-2005 for beef burger, the criteria of” EOS” No 1973-2005 for kofta, the criteria of “EOS” No-1972-2005 for sausage, the criteria of ‘EOS” No1114-2005 for luncheon.

PCR results obtained in table (4), showed *stx1* and *stx2* detected in O55:H7,only *stx2* detected in O78, while shiga toxin 1(*stx1*) and *stx2* detected in O119:H6, also O127:H6 positive *E.coli* strains for *stx1* ,*stx2* and *eaeA* genes but O124 negative *E.coli* strain for (*stx,1 stx2 and eaeA*) genes and O146:H21 only have *stx2* gene. these results nearly agree with (Fantelli and Stephan, 2001) isolated five different serotypes of the seven strains of *E. coli* two STEC strains harbored *stx1* and *stx2* and five strains harbored *stx2* genes also none of the strains was positive for *eae* gene.and agree with (Badri *et al.*, 2009) stated that PCR showed 37% (74) of *E. coli* isolates carried one or more of these virulence genes(included *stx1, stx2, eaeA, lt, st, hlyA, aggA, saa, astA, iucD and cnf1*) while (Elbagoury *et al.*, 2016) stated that *E.coli* O119 that proved to have Stx1 and Stx2 genes. *E.coli* O128 and O121 had only Stx1, while *E.coli* O146 had only Stx2.

Human infections caused by Verocytotoxigenic *E.coli* with lower incidences of 1.2 cases per 100,000 population. Meat and meat products are important sources for these infections but knowledge on exactly how important they are compared with other types of food, drinking water and environmental exposure is quite limited. Occurrences of zoonotic pathogens in raw meat are variable, although most often are between 1% and 10%, depending on the organism, geographical factors, farming and/or meat production practices. (Nørrung and Buncic ,2008) .

CONCLUSION

From the achieved results it can be concluded that: (1) Sausage and kofta were more

contaminated with the highest level of E.coli then luncheon follow by beef burger finally minced meat. (2) PCR assay is rapid, more specific, more sensitive and enables detection of food borne pathogen and their virulence genes.

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