MOLECULAR DETECTION OF SOME VIRULENCE GENES OF *E.COLI* ISOLATES FROM MEAT AND MEAT PRODUCT IN PORT-SAID GOVERNORATE

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ABSTRACT

Food stuffs of animal origin may present hazards, due to bacterial contamination. This study was conducted to determine the prevalence, serotypes, and virulence genes of *Escherichia coli* in raw beef meat and some of meat products (raw, minced meat, beef burger and sausage, 25 sample each). Bacteriological examination of total 100 samples were collected from different localities in port-said City showed that 10/100 (10%) of samples were infected with *E. coli*. The prevalence of *E.coli* strains in fresh and minced meat, beef burger and sausage samples were 4% (1/25), 16 % (4/25), 8 %(2/25), and 12% (3/25), respectively. Serological typing were conducted on 10 isolates and results obtained revealed that two (20%) O114, 2 (20%) O157, three (30%) O125, and one (10%) for each of O127; O18, and O158. Ten isolates were submitted to molecular studies for detection of the *traT* (transfer protein), *iutA* (encoding aerobactin) and *iss* (increased serum survival protein) genes, by using PCR technique. PCR assays for different virulence genes showed that sex (60%), four (40%), and sex (60%) strains carry *iss, iutA*, and *Trat* genes, respectively.

Keywords: E.coli, virulence genes, Meat, Meat Products, PCR.

INTRODUCTION

Meat and its products as essential for human survival, it also essential for microorganisms to grow and thrive as raw meat and meat products still threaten human health via harboring pathogenic and zoonotic *E. coli*, (Karmi 2019). Therefore, the protection of human health is important to understand the microbial hazards in foods. *E. coli* are found in warm-blooded species including humans as part of the normal intestinal flora (FSANZ, 2013, Sarwska

et al., 2019). They are facultative anaerobic, rod-shaped Gram-negative, bacteria it can grow at temperatures ranging between 7 – 46°C, pH of 4.4 – 10.0 and a minimum water activity of 0.95 when other conditions are near optimum (FSANZ 2013). There are over 160 serological types of *E. coli* which are involved in urinary tract infection, pneumonia, sepsis, surgical infection, gastrointestinal tract infections, hemolytic-uremic syndrome (HUS), and meningitis (Alkeskas *et al*., 2015). Although *E. coli* induced illness is much lower than that of other foodborne pathogens the severity of symptoms and the infectious dose made *E. coli* one of the most significant pathogens of public health importance, (Buncic, 2006, Lacher *et al.,* 2016).The Contamination may

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occur directly through animal feces or indirectly through employees or contaminated working aids (Gyles, 2007, Darwish *et al.,* 2018).

Meanwhile, the use of proper hygienic measures in handling food of animal origin and proper handling during processing and good heating before consumption are important control measures for the prevention of *E. coli* infections (Michael, 1991). Much is now known about the characteristics of this organism as it can survive in ground beef at − 20 ° C for nine months. The organism is heat sensitive, Proper cooking temperatures of 71 ° C will destroy the organism in foods. And it is quite salt tolerant, with the ability to grow at 8% NaCl at 37 ° C; however, at a lower incubation temperature of 10 ° C, growth was inhibited from 4% to 6%. (Toldrá, 2010).

Processed meats and ready-to-eat food supply the increasing demand for meals prepared simply and quickly, in developed countries, people today are less prepared to spend the time required to cook a traditional meat meal, especially during the week. In addition to the effects of consumers' preferences, another force driving processed meats supply is their higher economic value (Wood, 2006). Meat products could be contaminated with pathogenic microorganisms from meat handlers during the processes of manufacturing, packing and marketing (Darwish *et al.,* 2018). Improper cooking, refrigeration or storage may lead to meat borne illness. Food-borne pathogens are the leading causes of illness and death in developing countries costing billions of dollars in medical care, medical and social costs (Fratmico *et al.,* 2005). On the other hand, the combination of salt and nitrites which used in many meat products may have an inhibitory effect on *E. coli*, (Casey and Condon, 2000).

The gaining of virulence genes is believed to provide an evolutionary pathway to pathogenicity, most strains of *Escherichia coli* are harmless commensals of mammals (Selander, R. K. *et al.*, 1987), but others are capable of causing either intestinal or extra-intestinal disease (Orskov, F. and Orskov, I., 1992). Manifestation of clinical symptomology and pathology appears to be closely associated with the possession of certain virulence gene combinations in *E. coli* (Grauke, L.J., 2002). The virulence factors, such as adhesions, invasions, toxins, iron acquisition systems (siderophores), and protectins, are involved in colonization, adhesion, invasion, and survival against host defenses (Janßen *et al.,* 2001; Jeong *et al.*, 2012 and Navaro-Garsia *et al.,* 2019).

Therefore the present study was planned to detect the prevalence of *E.coli* in examined meat and some meat products, and detection of virulence genes in the isolates by using polymerase chain reaction (PCR).

MATERIALS AND METHODS

1-Collection of samples:
One hundred random samples of meat and meat by products as, minced meat, sausage and beef burger were randomly collected from markets in Port Said governorate. The collected samples were taken aseptically and transferred immediately to the laboratory in the (Department of Microbiology Port-Said lab). All samples were examined bacteriologically for detection of *E.coli*

2-Isolation and identification of *E. coli:*
The standard protocol described by (APHA, 2001) was adopted for the isolation of *E.coli* from meat samples by add 25 g of each sample to 225mL of buffered peptone water. This mixture was incubated at 37 ℃ for 18- 24 hours, the enrichment cultures were streaked on Eosin Methylene Blue (EMB) (Oxoid), 37℃ for18 -24 h, typical *E. coli* colonies were picked and inoculated in Nutrient agar for purification, identification, and serotyping.

3- Detection of virulence genes:

Is performed at (Reference laboratory for veterinary quality control on poultry production, Animal Health Research Institute) according to Yaguchi *et al.* (2007) and Kaipainen *et al.* (2002) as show in table (1)

DNA extraction: from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56ºC for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer: Primers used were supplied from Metabion (Germany) are listed in table (1).

PCR amplification: Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template.

Analysis of the PCR Products. The products of PCR were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the PCR products were loaded in each gel slot. Gelpilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table 1: Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondar denaturation	Annealing	Extension		
Iss	ATG TTA TTT TCT GCC GCT CTG	266	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Yaguchi *et al.*, 2007**
	CTA TTG TGA GCA ATA TAC CC							
iutA	GGCTGGAC ATGGGAAC TGG	300			63˚C 30 sec.			
	CGTCGGGA ACGGGTAG AATCG							
TraT	GATGGCTG AACCGTGG TTATG	307			55˚C 30 sec.			**Kaipain en *et al.*, 2002**
	CACACGGG TCTGGTAT TTATGC							

RESULTS

E. coli is a Gram negative, rod shaped bacteria under microscope, facultative anaerobic bacteria. On Eosin Methylene Blue colonies are metallic sheen green colonies. Several biochemical tests used for biochemical identification, It was positive for Indole, Methyle red, nitrate reduction, catalase, and negative for Oxidase, Urease, H2S, Simmon`s citrate, Voges- Proskauer, and gelatin liquefaction. *E.coli* ferment lactose and glucose with acid and gas production. *E.coli* was isolated from meat (1/25, 4%), minced meat (4/25, 16%), sausage (3/25, 12%) and beef burger (2/25, 8%). The total percentage of positive samples was 10%. The highest isolation percentage was from minced meat (16%) and the lowest percentage was from raw meat (4%). As shown in Table (2). Ten *E.coli* isolates were recovered from a total of 100 samples (meat, minced meat, sausage and beef burger) 25 each.

Table 2: Prevalence of *E. coli* in meat and meat products.

Type of samples	Number of samples	*E. coli* isolates		
		No.	%* N=25	%** N=100
Meat	25	1	4	1
Minced meat	25	4	16	4
Sausage	25	3	12	3
Beef burger	25	2	8	2
Total	100	10	40	10

*No. of isolates/ no. of samples.   ** No. of isolates/ total no. of samples.

Table 3: Serological identification of isolated *E. coli* from meat and meat products.

E.coli isolates	monovalent
Meat	O18
Minced meat	O114
Minced meat	O158
Minced meat	O127
Minced meat	O125
Sausage	O157
Sausage	O157
Sausage	O125
Beef burger	O114
Beef burger	O125

Ten *E.coli* isolates were submitted to serology unit – Animal Health Research Institute, for serological identification. The one *E.coli* isolate of meat was belonging to O18, the four *E.coli* isolates of minced meat were serotyped as O114, O158, O127 and O125, while the three *E.coli* isolates of sausage were O157, O157 and O125. The two *E.coli* isolates recovered from beef burger were O114 and O125, as shown in (Table 3).

Isolated *E.coli* (ten isolates) were submitted for molecular detection and determination of virulence genes: the Increased Serum Survival gene (*iss*), the Iron Uptake gene (*iutA*) and surface exclusion protein gene (*traT*) by RT-PCR, (Table 4). Figures (1, 2

and 3).The results showed that, *iss* gene was detected in 6 *E-coli* isolates, one from meat isolate, two form minced meat isolates, two from sausage isolates and one from beef burger. *iutA* gene was detected in 4 *E-coli* isolates, two from minced meat and two from sausage. *traT* gene was detected in 6 isolates, two from minced meat, two from sausage and two from beef burger, (Table 4).

Table 4: Prevalence of virulence genes (*iss, iutA* and *traT*) in *E-coli* isolates:

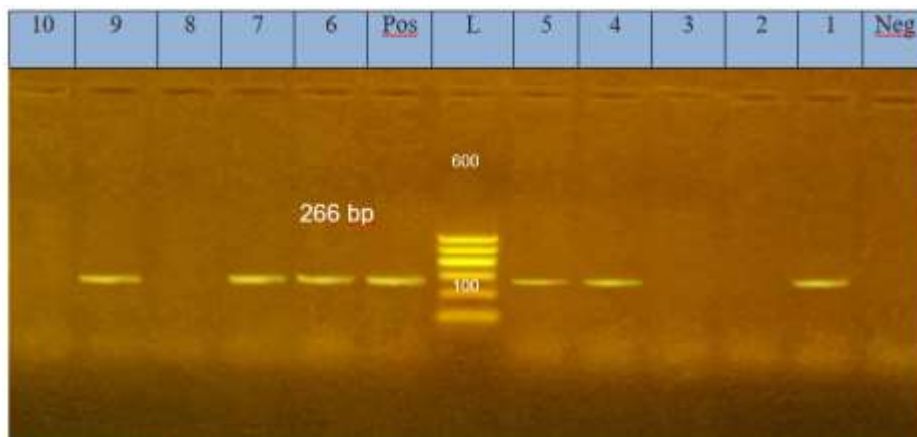| *E-coli* Isolate	Source	Results		
		Iss	*iutA*	*traT*
1	Meat	+	-	-
2	Minced meat	-	-	-
3	Minced meat	-	-	-
4	Minced meat	+	+	+
5	Minced meat	+	+	+
6	Sausage	+	+	-
7	Sausage	+	+	+
8	Sausage	-	-	+
9	Beef burger	+	-	+
10	Beef burger	-	-	+

Fig. (1): Agarose gel electrophoresis of amplified *iss* gene PCR product (266 bp).
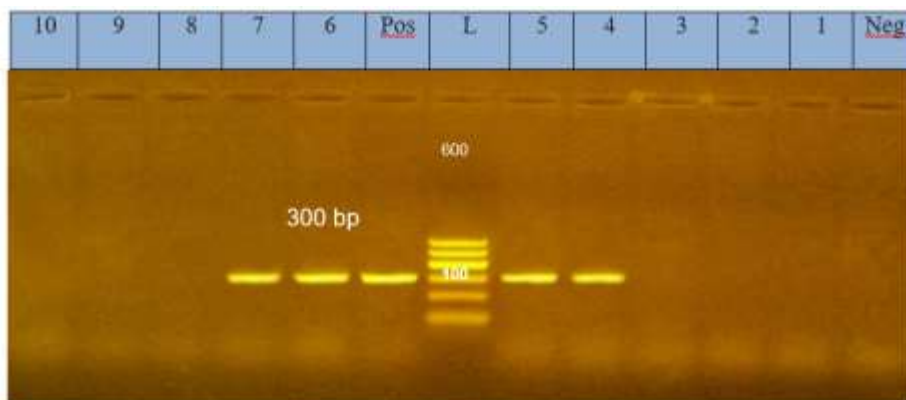

Fig. (2): Agarose gel electrophoresis of amplified *iutA* gene PCR product (300 bp).
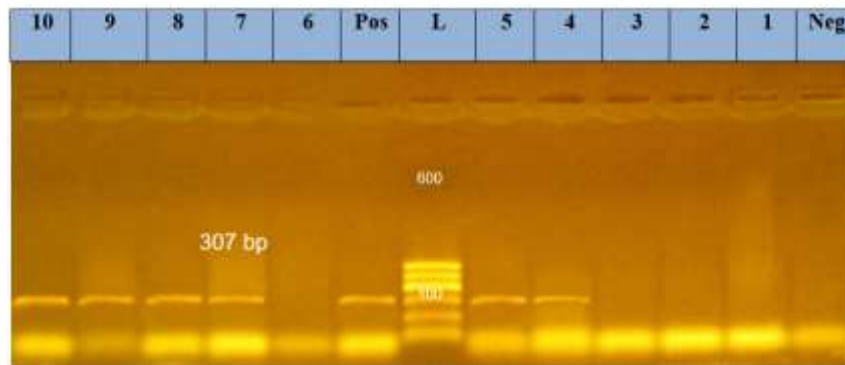
5

Fig. (3): Agarose gel electrophoresis of amplified *traT* gene PCR product (307 bp).

DISCUSSION

In the last years and due to great development in human life caused a great demined of easily prepared meals contained high level of animal protein. So meat and meat products are good source of protein contain an essential amino acids (Bieslski, 2005). The incidence of *E.coli* in the present study in table (2) show that ten isolate from 100 sample 10% positive of *E,coli* in meat and meat by product (minced meat, sausage, beef burger) which similar to Mohamed (2012) (9.3%) but less than obtained by Eid *et al.* (2019) in Port-Said who found it 43% the incidence of *E.coli* in fresh meat sample (4%)(1/25) which similar to Sallam *et al.* (2013) (3.7%) this result less than obtained by Zohreh Mashak (2018) and Ateba & Mbewe (2011) who found that the percentage of *E. coli* in meat are 14% and 27% respectively. Unlike fresh beef, which showed low prevalence for *E. coli* a higher contamination rate of 16% (4/25) was detected in the ground beef analyzed. In a previous study from Egypt, Abdul-Raouf *et al.* (1996) detected *E. coli* in ground beef at a lower occurrence of 6% (3/50)but our result similar with obtained by Ibrahim *et al.* (2015) and less than Zakarya and Fouad (2013) who found *E.coli* in minced meat 25%. regard to *E.coli* in beef burger 2/25 (8%)which nearly similar to obtained by Ahmed (1992), and EL-sherif (2009) who recorded the incidence of *E.coli* as 6.6% and 10% and less than obtained by Ibrahim *et al.* (2015) which found *E. coli* in beef burger as 24% in regarded to sausage the incidence

was 2% which nearly similar to obtained by Zakarya and Fouad (2013) and Ahmed (1992) were 16.6% and 15% respectively and lower than obtained by Ibrahim *et al.* (2015) and EL-Mossalami (2003) were 44% and 40% respectively. The higher contamination rate of *E. coli* strains in ground beef, sausage and beef burger samples in comparison to fresh beef samples in this study may be attributed to the fact that these products needs production process which may lead to contamination.

Out of 10 *E. coli* strains, only 6 were serotyped by using commercially available antisera as shown in Table (3). Distribution of 6 *E. coli* serotypes recovered from meat, minced meat, Sausage and beef burger were belonged to (O18, O114, O158, O127, O125, and O157) Similar findings have been reported by (Ibrahim *et al.,* 2015) who isolated *E. coli* O114, and O125 from meat, minced meat, burger and sausage, (Seran *et al.,* 2012) isolated O:157 K:- serotype from meat. Also Eid *et al.* (2019) isolated O114, O158, O127, O125. Karmi (2019) O157, O127, O125 from meat and its products. The most important *E.Coli* serotypes which have been associated with human illness are O157, O111, O26, O103, O113, O91, O117, O118, O121, O145, O128, and O146 (Rahal *et al.,* 2015), while Kaper 1994 proved that O125 and O127 are usually transmitted by contaminated food and colonize the small intestine where they attach tightly to the epithelial cells of the villus tips and cause typical lesions called attaching and effacing lesions.

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The difference in the rate of isolation of *E. coli* and its serotypes may attribute to difference in localities, methods of sampling and total number of samples. Also the variation in the results obtained by different investigators may be due to difference in manufacturing practices, handling and difference in time while Undercooked meat products have caused many food poisoning incidents associated with *E. coli* which is present in the faces, intestines and hide of healthy cattle from where it can potentially contaminate meat during the slaughtering process (Duffy *et al.,* 2003, Darwish *et al.,* 2018). *Enterobacteriaceae* were very useful as indicators of bad hygiene or bad treatment of food products and their presence in large number indicates a big possibility of the multiplication of *E.coli* and other pathogens (Nissen *et al.,* 2001).

While *E. coli* serotyping is an important technique for making the proper diagnosis and epidemiological investigations during food-borne outbreaks, it cannot be relied on alone for categorizing a strain of *E. coli*, so the identification of specific virulence genes must also be performed (Barlow *et al.,* 1999). PCR is a powerful molecular biology technique for the detection of virulence genes, it is not only highly sensitive and specific, but it also provides rapid and reliable results (Abdeltawab 2015). It can help to distinguish *E. coli* isolates from meat and meat products through detection of virulence genes (Kimata *et al.,* 2005). Our study revealed that *iss* gene was detected in 6/10 (60%) *E. coli* isolates while the highest result 100% recorded by Jørgensen *et al.* (2019), Oliveira *et al.* (2019*)* and Marwa Hassan *et al.* (2020) and the lowest 20%, 15.2 and 31.8 recorded by, Cyoia *et al.* (2015) and Bok *et al.* (2020) respectively. In the present work, it was found that the prevalence rate of *iutA* gene was 40% of the tested *E. coli* isolates, which nearly agree with lopez-banda *et al.* (2014), 48.1% the highest prevalence rate of *iutA* gene was detected by Hussein *et al.* (2013), Chalmers *et al.* (2017), and Jørgensen *et al.* (2019) in 100%, 93.3% and 96.9% of the *E. coli* isolates, respectively. While Eftekharian *et al.* (2016), detected *iut*A gene in 70% and lopez banda *et al.* (2014) in 50% of

E. coli isolates. The virulence gene *(traT) detected* in 60% of isolates which nearly to Bok *et al.* (2020) 54.9% while 88.8% and 88.1% detected by Marwa Hassaen *et al.* (2020) and *Kogovšek et al.* (2019). While, 47.7% detected by Ferreira *et al.* (2018). Jiménez *et al.* (2020) determined *traT* gene in 17 isolates of *E.coli* 4, from beef, 1 from pork, chicken, wiled fox and 10 from human.

The importance of *iss* function that allows *E. coli* to avoid host defenses, multiply and distribute, thus promoting the development of the disease (López *et al.,* 2017). The *iut*A gene is one of the 5 genes of the aerobactin operon. It encodes an outer membrane protein involved in the high-affinity binding of Fe^{+3} aerobactin and can be plasmid located (Johnson *et al.,* 2006) or chromosomally encoded in some strains (Schouler *et al.,* 2012). The virulence gene *(traT)* outer membrane protein serum resistance that codifies an outer membrane protein implicated in serum survival, (Johnson & Stell, 2000), the *EXPE* constitute *(traT)* gene more commonly than commensal strains Cyoia *et al.,* 2015. The variation in the presence of virulence encoding genes among different serotypes isolated from different sources revealed that the mechanisms of pathogenesis depends mainly on the presence of different virulence factors not to the different serotypes. (Aranda *et al.,* 2004).

Results obtained showed that marketing meats, especially, minced beef, were contaminated with *E.coli* strains harboring some virulence genes these findings suggest that, under certain conditions as inappropriate meat handling, consumption of undercooked meat, the possibility exists for retail meat to serve as a vehicle for transmitting pathogenic *E.coli* to consumers.

CONCLUSION

Results of this study revealed the presence of *E. coli* in raw meats and some its products reaching consumers, indicating possible risks of infection to people through the consumption of raw/under-cooked meat or cross-contamination of other food products. Coordinated actions are needed to reduce or eliminate the risks posed by this organism at various stages in food chain.

2- In addition to the conventional methods used for isolation and identification of *E. coli*, PCR is required as rapid, accurate and specific tool for detection of pathogenic *E. coli* and their virulence genes.

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" الكشف الجزيئي عن بعض جينات الضراوة لعترات الايشيريكية القولونية المعزولة من اللحوم ومنتجاتها في محافظة بورسعيد"

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استهدفت هذه الدراسة إلقاء الضوء على مدي تواجد ميكروب الايشيريشيا كولاي في اللحوم و بعض منتجاتها وتحديد بعض جينات الضراوه بها والذي قد يمثل تهديدا لسلامة الاغذيه لذلك تم فحص عدد ١٠٠ عينه من اللحوم و منتجاتها [لحم طازج ,لحم مفروم , سجق , بيف برجر] (٢٥ عينه من كل منها) تم تجميعهم عشوائيا من السوبرماركت و محلات الجزاره في محافظة بورسعيد و أظهرت نتائج الفحص البكتريولوجي و البيوكيميائي أن عدد ١٠ عينه (١٠%) كانت موجبه لميكروب الايشيريكية القولونية, وكانت نسبة الأصابة في كل من اللحم و اللحم المفروم والبيف برجر والسجق(٤و١٦و٨و١٢%)على التوالي كما أوضحت نتائج التصنيف السيرولوجي لميكروب الايشيريكية القولونية المعزول من عينات اللحم و اللحم المفروم و البيف برجر والسجق أن عترات الايشيريكية القولونية كانت تنتمي الى مجموعات O :125, O:18, O:114, O:158, O:127, O:157 و باستخدام اختبار تفاعل انزيم البلمرة المتسلسل ذو الوقت الحقيقى لتحديد جينات الضراوه ، أسفرت النتائج ان ميكروب الايشيريكية القولونية (المعزوله من اللحوم ومنتجاتها أظهرت تحديد جينات الضراوه iss, traT , iutA. والتى تؤثر على سلامة الغذاء مما يشكل خطورة على المستهلكين.