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
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), andprotectins, 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 °C for 18-24 hours, the enrichment cultures were streaked on Eosin Methylene Blue (EMB) (Oxoid), 37°C 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 56°C 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.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primers sequences</th>
<th>Amplified segment (bp)</th>
<th>Primary Denaturation</th>
<th>Amplification (35 cycles)</th>
<th>Final extension</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iss</strong></td>
<td>ATG TTA TTT TCT GCC GCT CTG CTA TTTG TGA GCA ATA TAC CC</td>
<td>266</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec. 54°C 30 sec. 72°C 30 sec. 72°C 7 min.</td>
<td></td>
<td>Yaguchi et al., 2007</td>
</tr>
<tr>
<td><strong>iutA</strong></td>
<td>GGCTGGGAC ATGGGAAC TGG CGTCGGGA ACGGGTAG AATCG</td>
<td>300</td>
<td></td>
<td>63°C 30 sec.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TraT</strong></td>
<td>GATGGCTGT AACCCTG TTTATG CACACGGG TCTGGTAT TTATGC</td>
<td>307</td>
<td></td>
<td>55°C 30 sec.</td>
<td>Kaipainen et al., 2002</td>
<td></td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Number of samples</th>
<th>E. coli isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Meat</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Minced meat</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Sausage</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>Beef burger</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>10</strong></td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>E. coli isolates</th>
<th>monovalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>O18</td>
</tr>
<tr>
<td>Minced meat</td>
<td>O114</td>
</tr>
<tr>
<td>Minced meat</td>
<td>O158</td>
</tr>
<tr>
<td>Minced meat</td>
<td>O127</td>
</tr>
<tr>
<td>Minced meat</td>
<td>O125</td>
</tr>
<tr>
<td>Sausage</td>
<td>O157</td>
</tr>
<tr>
<td>Sausage</td>
<td>O157</td>
</tr>
<tr>
<td>Sausage</td>
<td>O125</td>
</tr>
<tr>
<td>Beef burger</td>
<td>O114</td>
</tr>
<tr>
<td>Beef burger</td>
<td>O125</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>E-coli Isolate</th>
<th>Source</th>
<th>iss</th>
<th>iutA</th>
<th>traT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Meat</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Minced meat</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Minced meat</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Minced meat</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Minced meat</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Sausage</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Sausage</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Sausage</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Beef burger</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Beef burger</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**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).
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) showed 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.
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 iutA 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 Hassaan 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 iutA 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.

REFERENCES


