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RESEARCH ARTICLE **(Open Access)**

Occurrence of *E. coli* O:157 H:7 of ground meat samples collected from butcher shops in Tirana market

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Abstract

This study is carried from 2011 to 2012 regarding to detection of E. coli O157 H:7 in ground meat samples collected from butcher shops in Tirana. 83 ground meat samples collected are analyzed for presence of E. coli O157 H:7 performing standard anlytical procedures. Study results confirmed the positive cases of *E. coli* in average value 7.2.% (6/83). 4 isolates or 4.8% (4/83) of ground meat samples was identified by serological tests as E. coli O157H:7. Sanitary conditions of meat processing and manipulation in meat shops referred to our study results are not at the level that requires domestic legislation and don't accomplish EU criteria for processing meat and selling it in Tirana market.

Key words: *E. coli O:157H: 7*, ground, meat, market, Tirana

1. Introduction

E. coli live in the intestines of humans and animals. There are strains of E. coli known to produce toxins that can cause diarrhea. *E. coli* strain called O157:H7 can cause severe diarrhea and kidney damage. *E. coli* live in the intestines of healthy cattle, and contamination of the meat may occur in the slaughtering process or processing. [**1**, **5**]. Food infection can also occur after consuming foods such as minced meat, salami, and unpasteurized milk, juice or other kinds of food. Other source of contamination is person-to-person transmission occuring if infected people do not wash their hands after using the toilet. *E. coli* O157:H7 is markedly different from other pathogenic *E. coli*, as well [**5**, **16**]. In particular, the O157:H7 serotype is negative for invasiveness adheres through the *E. coli* common pilus (ECP), and does not produce heat stable or heat labile toxins [**4**, 12]. In addition, *E. coli* O157:H7 is usually sorbitol negative, whereas 93% of all *E. coli* ferment sorbitol. *E. coli* O157:H7 also lacks the ability to hydrolyze 4-methylumbelliferyl-β-D-glucuronide (MUG) and does not grow at 45 °C in the presence of 0.15% bile salts [**2**, **8**]. Because of such characteristic, this serotype cannot be isolated by using standard fecal coliform methods that include incubation at 45 °C. *E. coli* O157: H7 serotypes are closely related descended from a common ancestor. Plasmids content with more than chromosomal content are no more related to other shiga toxin [15, **16**]. *E. coli* O157:H7 are most closely related and diverged from a common pathogenic ancestor that possessed the ability to form attaching and effacing lesions. *E. coli* O157:H7 serotypes apparently arose as a result of horizontal gene transfer of virulence factors. Some kinds of *E. coli* cause disease by making a toxin called Shiga toxin. The bacteria that make these toxins are called "Shiga toxin-producing" *E. coli*, or STEC for short [**7**, **16**]. The most commonly identified STEC in North America is *E. coli* O157:H7 (often shortened to *E. coli* O157 or even just "O157"). These other kinds are sometimes called "non-O157 STEC." *E. coli* serogroups O26, O111, and O103 are the non-O157 serogroups that most often cause illness in people in the United States [**3**, **16, 17**]. Exposures that result in illness include consumption of contaminated food, consumption of unpasteurized (raw) milk, consumption of water that has not been disinfected, contact with cattle, or contact with the feces of infected people. Some foods as meat, meat processed products are considered to carry such a high risk of infection with *E. coli* O157 [**2**, **6**, **9**]. People have gotten infected by swallowing lake water while swimming, touching the environment in petting zoos and other animal exhibits, and by eating food prepared by people who didn't wash their hands well after using the toilet. Almost everyone has some risk of infection [**2**, **4**]. New data released by the CDC show that *E. coli* O157:H7 infections have declined 51% since 2000 [**3]**. The incidence of *E. coli* O157:H7 infections in Americans dropped from 1.12 cases per

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100,000 people in 2008 to 0.99 cases per 100,000 people in 2002 according to data of CDC in 2004. This represents an overall 51 percent reduction since 2000 achieves the United States Healthy People 2010 objective for *E. coli* O157:H7 infections [**2**, **7, 17**]. AMI Foundation study done in five packing plants showed that while 18% of incoming cattle tested positive for *E. coli* O157:H7 on their hides, no carcasses tested positive for the pathogen following careful hide removal and a series of antimicrobial treatments [**3**, **16**].

2. Materials and method

Ground meat samples were collected from diffrent market places in Tirana. Analytical method was based on standard method (ISO 1665, 2001). For each sample were collected 5 units representing 1 kg ground meat. Each samples was registred and filling out accompanied document. The prformed method was based on qualitative determination of the presence of pathogenic *E. coli*. Aseptically weigh 25 g of sample into 225 ml of BHI broth (dilution factor of 1:10). If necessary, sample size may deviate from 25 g depending on availability of the sample, as long as the diluent is adjusted proportionally. Sample was blended or stomached briefly. Incubation of the homogenate for 10 min at room temperature with periodic shaking was performed and then was allowed the sample to settle by gravity for 10 min. Medium was decanted carefully into a sterile container and incubation was carried out for 3 h at 35°C to resuscitate injured cells. Transferring of contents to 225 mL double strength TP broth in a sterile container and incubation at 20 h at 44.0 ± 0.2°C was further step of laboratory procedure. After incubation, the culture was streak to L-EMB and MacConkey agars and agars plates were transferred for incubation for 20 h at 35°C [**5**]. The positive selected culture of E. coli was transferred for further identification to be tested for O157:H7 were enriched in EHEC Enrichment Broth (EEB), plated on TCSMAC to isolate sorbitol non-fermenting colonies, which were then identified phenotypicall and serologically. Confirmation of presumptive positive results was completed within 3 days. A multi-laboratory validation study showed that this method was superior to the EEB/TCSMAC method for the recovery of *E. coli* O157:H7 bacteria in artificially contaminated foods [5, 16]. This screening method uses modified Buffered Peptone Water with pyruvate (mBPWp), which contain several anti-microbial reagents that effectively suppress normal flora growth and non-target competitors, yet allows the growth of viable O157:H7 cells (including other STEC) and is capable of detecting <1 cfu/g in foods. Further identification was completed by carrying out serological tests using as material colonies selected from CT-SMAC medium [8].

Table 1: Isolates of E. coli tested with antiserum O157 and flagellum antiserum H: 7.

Isolates of E. coli from ground meat samples	*Positive-tested with antiserum O157*	*Positive-tested with flagellum antiserum H7*
6	4	4

Table 2: Incidence of *Escherichia coli* O157:H7 strains isolated and identified controlling positive of ground meat samples collected from 2010 to 2011.

Ground meat samples	*Positive samples evaluated in cfu/g E. coli*	*Positive cases with Escherichia coli O157:H7*
83	6/83 (7, 2%)	4/83 (4.8%)

3. Results

In this study is carried out analytical control for enumeration and identification of *E. coli* of 83 ground meat samples collected in butcher shops in Tirana market. The other objective of this study was isolation and identification of group of strains of *E. coli* O:157 H:7. All starins isolated and idenrtified as *E. coli* from ground meat samples was tested with API 20 E. The biochemical feature was completed and all strains were tested with additional tests for identification of *E. coli* O:157 H:7 transferring isolates into MaCconkey Sorbitol Agar (CT-SMAC) plates. According to Table 1 after that serological identification with specific antisera of *E. coli* O:157 H:7 only 4 strain or 4,8% are confirmed positive. From 2010 to 2011 are analyzed 83 groundd meat samples confirmed presence of *E. coli* in 6 samples or 7.2% of total samples.

4. Discussion

Identified bovine minced meat samples shows that these products until the final preparation phase spend some manipulation. From the results obtained are shown in the above tables 1 amd tanles 2 presence of *E. coli* in the samples of minced meat is confirmed in (1) gound meat from chicken and (5) minced beef cattle. According to Table 2, 7.2 % of checked samples are confirmed by the value of *E. coli* higher than permissible limit [**14**]. Even in some European countries results of some studies are reported increased values of *E. coli* in 11-14% of controlled samples [**1**, **8**, **16**]. These countries apply strong measures and sanitary preparation of minced meat turns out that incidence to be lower. Samples identified in our case with high values indicate that the sanitary conditions during their preparation were not the right level. Environments in which minced meat prepared and stored deemed inappropriate and bring increased levels of microorganisms [**5**, **7**]. The values found beyond the limits recommended in the specific regulations [**14**] where allowed values 50-500 cfu/g. Minced meat is good medium for growth of microorganisms during preparation and in this way is compromising the safety of this product [**1**, **4**, **10**]. Minced meat can't be kept more than 2 days in cold temperatures, which should be 0-4^{o} C. Maintaining higher temperature cause adding of *E. coli* progressively. Preservation of frozen and packaged makes it sure though minced meat does not protect from oxidation processes which deal with the change of color in purple or brown. Different authors emphasize that "shell life" favors the growth of strains of *E. coli* O157: H7 [**4**, **12**, **16**]. In our study was done identification of strains of this group with the above mentioned method. Analogue studies show a positive correlation between the number of *E. coli* found in an animal product and the presence of *E. coli* O157: H7 [**7**, **8**, **12**, **16**]. This fact indicates that even in our case the possibility of contamination exists in 4 controlled samples. In 6 positive samples (Table 1) was evaluated numeric identification and only 4 samples were identified with *E. coli O157: H7* or 4.8% of the total of analyzed minced meat samples. Mixture of minced meat with other ingredients in the case of preparing meatballs, and sausages and kimas or hamburger in the first moments is associated with increased number of *E. coli*. In the case of fermentation processes acid environments reduces the number of *E. coli*. This was reported from various studies which show that *E. coli* number was reduced 2 or 3 times. Reducing this kind of contamination or ensuring low incidence toxic-infections of minced caused by *E. coli* O157: H7 required application of the principle of keeping everything clean during manipulation with minced meat. In the fermented minced meat used for sausage production, *E. coli* O157: H7 is reduced because the pH achieves values from 4.6 to 4 [**3**, **5**, **13**]. The contamination also is influenced by other factors like the presence of humidity of minced meat, pH, free water activity and temperature of storage. Today in our country is not known how many infections caused by *E. coli*. It is also natural that the incidence caused by minced meat is unknown. Despite occasional studies, accurate information will be available collecting data from cases of these intoxications [**9**, **12**]. Many developed countries have undertaken programs of several years of monitoring of food intoxication caused by *E. coli* O157: H7. Major research centers has as study subject strains of this group which are today one of the most pressing problems of food safety [**11**, **12**]. Considering the numerical evaluation of *E. coli* very important in analytical control of minced meat is always recommended that this indicator should be conducted for analytical of food samples involved in the studies. Number of *E. coli* per unit weight or volume used to be as an indicator of microbiological contamination of minced meat. This should sufficient to identify different strains remains crucial for determining the risk that is present in this type of food product. In cases of employment of workers in meat processing facilities required information on the history of their health status is important because food infections may have transmitted to the persons who shall be employed. In this case they could be sick having a toxic food-infection caused by *E. coli* O157: H7, so they are required to perform microbiological analysis of control again.

5. Conclusions

- From analytical control is confirmed that 7.2 % of the controlled samples were containing levels of *E. coli* above the acceptable level. Minced meat sold in meat shops in Tirana market is real health risk for consumers.
- Identification of serological groups *E coli* O157: H7 in minced meat samples was achieved. This kind of pathogen was isolated from control performed finding out 4 positive samples representing 4.8 % of ground meat samples in the study.

- Sanitary conditions of meat processing and manipulation in meat shops referred to our study results are not at the acceptable level according to domestic legislation and don't accomplish EU criteria for processing meat.

6. References

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