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A Study on Prevalence of *Escherichia coli O157* with a Verified Method in Foods

Semiha Yalçın[1*], Ayla Ünver Alçay[2], Gözde Yüzbaşıoğlu[1], Burcu Çakmak[3], Aysun Sağlam[4]

[1]*Marmara Food Analysis Laboratory, Istanbul Aydin University, 34295 Istanbul, Turkey*
[2]*Department of Food Technology, Istanbul Aydin University, 34295 Istanbul, Turkey*
[3]*Agrolab Food Analysis Laboratory, 34394 Istanbul, Turkey*
[4]*Department of Food Quality Control and Analysis, Istanbul Aydin University, 34295 Istanbul, Turkey*

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[*]Corresponding Author:
E-mail: semihayalcin@aydin.edu.tr

ABSTRACT

The purpose of this study were to identify the presence of *E.coli O157* and to determine its prevalence in foods which were collected from various restaurants, shops and markets in Istanbul. Also, validation of detection method of *E. coli O157* in all food stuffs was carried out according to applicability, repeatability, and minimum detection limit (LOD) and false positive and negative analysis based on TS EN ISO 16654 standard method. The results showed that the prevalence of *E. coli* O157 in food was 2%, and its prevalence increased in April and May.

Introduction

Escherichia coli is a bacterium that normally presents in the intestines of humans and warm-blooded animals. Most of its strains are harmless and actually are necessary for human intestinal flora. However, pathogenic strains of *E. coli* cause serious infections in humans and animals. These pathogenic variants of *E. coli* can be transmitted by mainly contaminated meat and meat products, raw milk, fruits, vegetables, water and environmental resources (Anonymous, 2011; Anonymous, 2014).

Escherichia coli belongs to *Enterobacteriaceae* family. There are six of *E. coli* pathotypes cause immense outbreaks in the world, such as Enteropathogenic *E. coli* (EPEC), Shigatoxin or Verotoxin producing *E. coli* (STEC/VTEC), Enteroinvasiv *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffusely Adherent *E. coli* (DAEC), Enterotoxigenic *E. coli* (ETEC) and a new pathotypes identified as adherent-invasive *E. coli* (AIEC). STEC/VTEC is also known as Enterohaemorrhagic *E. coli* (EHEC) (Anonymous, 2014; Croxen et al., 2013).

Verotoxin producing *E. coli* (VTEC) have been identified for the first time in 1970's by Konowalchuk and co-workers in Canada (Konowalchuk et al., 1977). The lethal toxin for HeLa cells in the polypeptide structure identified by O'Brien and LaVeck in 1983 was obtained from strains of verotoxin producing *E. coli*. Toxin is called shiga-like toxin because of similarity in its structural and antigenic characteristics with Shigella dysenteriae type 1 toxsin (O'Brien et al., 1983).

Verotoxin producing *E. coli* (VTEC) is an important pathogen that present in foods and water resources, which causes epidemic diseases such as diarrhoea, haemorrhagic colitis and haemolytic uremic syndrome. There are over 380 OH serotypes of *E. coli* (VTEC). Nevertheless, serotypes which cause serious infections in humans are limited. O26, O91, O103, O111, O113, O121, O145 and O157 serotypes are important infection treats. Among those, the most common infection causing serotype is *E. coli O157*:*H7* serotype. Therefore, VTEC serotypes are classified as *E. coli O157* and non-*E. coli O157*. *E. coli O157* is called Enterohaemorrhagic *E. coli* (EHEC) (Karmali et al., 2010).

Symptoms in the EHEC infection are severe abdominal pain and bloody diarrhea. Usually not exceeding 38.5°C fever and vomiting may occur. The haemolytic uremic syndrome (HUS) can develop at more than 10% of patients, and can result in death of 3-5% of these cases with the HUS table. It can be seen acute renal failure in cases of children. The symptoms of the disease

arise in 3-4 days after exposure, after average 7 days. The incubation period may vary between 1-10 days. Various antibiotics are used for the treatment (Anonymous, 2011; Anonymous, 2014).

E. coli O157 can be detected by using PCR (Polymerase Chain Reaction) and immunospesific methods; classical serological and culture methods are also commonly used in laboratory (Karmali et al., 2010).

In this study, prevalence of *E. coli O157* in foods in Istanbul was performed by classical culture method which was verified according to TS EN ISO 16654. Repeatability, the limit of detection (LOD), false positive rate, false negative rate, specificity, sensitivity studies were evaluated for method verification (Eurachem Guide AML, Health Protection Agency Guidance Note (NHS), QSOP 22).

Materials and Methods

Method Verification Studies

Stock preparation and sampling: Samples were selected according to ISO 19036 as infant formula, mayonnaise, dog food and mold cheese. *E. coli O157* ATCC 700728 (CECT) strain was used as positive control, and *Enterococcus faecalis* ATCC 19433 (Liofilchem) was used as negative control according to ISO 11133 directive. Stock solution was prepared for artificial contamination of selected foods with *E. coli O157* ATCC 700728 strain. Mac Farland (Biomerieux) standard was used for determining microorganism number and then serial dilutions were prepared for obtaining 2 cfu/ml and 100 cfu/ml for revealing limit of detection and detection of high level microorganism. Studies were carried out by two analysts for ensuring reproducibility of the method.

Method: Samples were homogenized with sterilized blender. Homogenized sample was weighed 25 g in stomacher filter bag. Samples were artificially contaminated with *E. coli O157* ATCC 700728 and *Enterococcus faecalis* ATCC 19433 with using stock solution after that 225 ml tryptone soya broth (added novobiocin) (LabM) was added to sample for pre-enrichment at 41.5°C for 6 hours. For selection of pure form of *E. coli O157* from the pre-enrichment medium, immunomagnetic separation process was performed according to the Captivate™ O157 (Lab M-CAP001) protocol. After the separation process, 50 µl of sample was inoculated in Sorbitol Mac Conkey Agar (CT-SMAC) (LabM) that prepared with adding Cefixime and potassium tellurite. Also, it was inoculated in TC-SMAC agar. Colonies were incubated for 18-24 hours at 37°C. Typical colonies of *E. coli O157* were observed as transparent, light yellowish-brown and about 1 mm in diameter in TC-SMAC agar. 5 typical *O157* colonies were selected and inoculated to Nutrient Agar then incubated for 18-24 hours at 37°C. Pure cultures were obtained from nutrient agar to be used for biochemical identification such as oxidase, catalase tests and Gram reaction. Gram-negative, bacilli form, oxidase negative, catalase positive colonies were selected for biochemical identification. Biochemical characteristics of the bacteria were determined by GNA A-B biochemical kit (Microgen) and *E. coli* was identified. For identification of *E. coli O157*, latex agglutination test (Microgen) was used according to the manufacturer instruction.

Repeatability, false negative rate, false positive rate and lod study: Analyses were carried out by two analysts for ensuring repeatability in same day at short intervals. 10 different sampling were performed for each of 4 different product groups (infant formula, moldy cheese, dog food and mayonnaise) which were artificially contaminated with *E. coli O157* at LOD level (2 cfu/25 g) and also at high level (100 cfu/25 g). False negative rate study was performed at these two levels as high and low. Artificially contaminated with *Enterococcus faecalis* samples were analysed for false positive rate study.

Prevalence of E. coli O157 Study

Isolation and identification of E. Coli O157 strains: Total of 200 samples were collected randomly in April 2015 and in January-May 2016 from 63 different food companies in Istanbul as shown in Table 1.

All analyses were carried out according to international standards TS EN ISO 16654 classical method.

Results

In this study, determination and identification of *Escherichia coli O157* was carried out according to verified TS EN ISO 16654 method in foods. Our test results showed that false negative and false positive analysis results were calculated as %0, relative sensitivity, relative accuracy and relative specificity analysis results were calculated as %100. Verification of TS EN ISO 16654 method study was evaluated as acceptable when interpreted in compliance with the Kappa (K) method (Table 2) (NMKL Procedure No. 20). Besides, for accuracy study, we participated to international proficiency test round for *E. coli O157* screening analysis. Proficiency test results were appropriate.

In this research, also *E. coli O 157* was screened in various food samples apart from the verification study. Nine samples were collected from different markets in 2015 April and two of them were positive for *E. coli O157*. *E. coli O157* was detected in 2 out of 10 samples picked from different food production areas in May 2016. In April 2015, raw chicken obtained from chicken shredding facility and ground beef samples obtained from a butcher were positive for *E. coli O157*. Among the samples collected in May 2016, raw beef mince and garnish were positive for *E. coli O157*. The prevalence of this pathogen in 200 food samples was determined as 2%.

Discussion

Food control laboratories have been authorized for detection of such pathogens and other elements in foodstuffs that risk public health by Ministry of Food, Agriculture and Livestock. This study was carried out in

accordance with international standards analysis for fulfilling the obligation for authorization process. Only accredited laboratories are authorized. In the accreditation process, after verification of all laboratory analyses, verification studies are attested by TURKAK (Turkish Accreditation Agency) according to international validity of the scope of TS EN ISO/IEC 17025 (Eurachem Guide AML, Health Protection Agency Guidance Note (NHS), QSOP 22, Turkish Food Codex Microbiological Criteria Regulation, 2011).

Table1 Collected samples

Sample	NS	Sample	NS
Chicken meat (raw)	5	Mayonnaise	1
Mediterranean salad	2	Meatballs with vegetable (raw)	1
Chicken meatballs (raw)	1	Meat ***doner with souce	1
Chicken schnitzel (raw)	1	Garnish	4
Chicken burger (raw)	1	Minced veal (raw)	36
Chicken nugget (raw)	1	**Lahmacun minced meat (raw)	2
Sheep lard (raw)	1	Minced lamb (raw)	4
Veal carcass (raw)	7	Part of lamb meat (raw)	5
Veal part of meat (raw)	17	Lamb carcass (raw)	3
Cubed veal (raw)	5	Veal liver (raw)	2
Cooked chicken ***doner	4	Sauce	1
Minced meat (raw)	6	Cake	1
Veal rib meat (raw)	1	Veal meat with souce (raw)	1
Cooked meat ***doner	1	Frozen red pepper	1
Meatballs (raw)	44	Frozen diced tomatoes	1
*Inegol meatballs (raw)	4	Veal tenderloin (raw)	2
Grilled meatballs (raw)	9	Veal arm (raw)	1
Hamburger meatballs (raw)	15	Lamb's shank (raw)	1
Shrimp (raw)	1	*Adana meatballs (raw)	1
American salad	1	Fish (raw)	1
Cucumber	1	Sausage	2
Total			200

NS: Number of sample, *A kind of Turkish traditional meatball. **A kind of Turkish fast food with very thin dough, like pizza. ***A kind of Turkish fast food.

Table 2 Results for kappa evaluation

Verification Samples	Positive Result	Negative Result	Total
E. coli O157 inoculated samples (positive samples)	160 (a)	0 (b)	160 (a+b)
Samples that not include *E. coli O157* (negative samples)	0 (c)	80 (d)	80 (c+d)
Total	160 (a+c)	80 (b+d)	240 (n=a+b+c+d)

a: E. coli O157 inoculated samples and positive identified samples, b:İnoculated samples but identified as negative, c: Not inoculated but identified as positive samples, d: Only Enterococcus faecal is inoculated but identified as negative samples, n: Total number of samples, Relative Sensitivity: a/(a+b)x100 = 160/160x100 = %100Ş Relative Accuracy: a+d / n x100 = 240/240x100 = %100, Relative Specificity: d/c+dx100 = 80/80x100 = %100, False negative rate: b/b+a = 0/160 = %0, False positive rate: c/c+dx100 = 0/80x100 = %0

E. coli O157 is a serious threat to public health in Europe and America that led to the outbreak. In our country, the Turkish Food Codex, Microbiological Criteria for food safety was published by the Ministry of Food, Agriculture and Livestock. According to this regulation; particularly meat and meat products, fermented meat products, fruits and vegetables, including processed products and ready to consumption food, *E. coli O157* certainly must not be present (Turkish Food Codex Microbiological Criteria Regulation, 2011).

The presence of *E. coli* in Turkey was demonstrated in many studies before. *E. coli O157* was investigated in 150 samples of calf minced and lamb minced meat collected from retail markets and butcheries and *E.coli O157* was found in 6.66% in total of 300 samples in Van (Alişarlı and Akman, 2004). And also in Afyonkarahisar *E. coli O157:H7* presence was shown in raw milk and cheese samples (Akkaya et al., 2007). Although registrations and information about prevalence and incidence of STEC strains are limited in Turkey (Bostan et al., 2005). In our study, 186 meat products and 14 salads including sauce samples were investigated for *E.coli O157* presence and detected in 3 meat products including 1 cooked meat sample and in 1 garniture sample.

In Turkey, *E. coli O157:H7* were not isolated from 100 pieces hamburgers (Sarımehmetoğlu et al., 1998). 127 samples of frozen hamburger and meatballs were screened for *E. coli O157:H7*, and *E. coli O157: H7* was isolated from 3 samples (O'Brien and LaVeck, 1983). On the other hand, in our study, *E. coli O157* was detected in raw chicken and raw mince samples and also cooked chicken, ready-to-eat garniture sample.

In Konya, sausage, salami, hamburger meat, Inegol meat ball, pastrami, minced meat and brisket and haunch of poultry were collected from different meat sellers, and analyzed for presence of *E. coli O157* and coliform

bacteria. *E. coli O157* (11.1%) was determined, in two of them *E.coli O157:H7* (8.1%) and in one refrigerated hamburger meat ball sample *E. coli O157* and *E.coli O157:H7* (4.34%) were determined (Balpetek and Gürbüz, 2010).

In another study in Turkey in Aydin, a study was carried out for detecting *Escherichia coli O157:H7* serotype in minced beef and uncooked beef burgers collected from butcher shops and supermarkets. *E. coli O157* serotypes were isolated from 6 minced beef and 6 uncooked hamburger meatballs by conventional culture techniques (Sezgin, 2013).

In Samsun, a total of 200 samples collected from cattle carcasses and the rectal contents of 100 slaughtered cattle from two commercial abattoirs were analyzed for *Escherichia coli O157* and *E. coli O157:H7* using immunomagnetic separation technique and multiplex PCR methods. *E. coli O157* and *E. coli O157:H7* were detected in 52 out of 200 samples (26%) tested. Of the positive samples, 49 were *E. coli O157* and three were *E. coli O157:H7*. The *stx1* and *stx2* genes were both detected in 35 *E. coli O157* isolates and one *E. coli O157:H7* isolate, but the stx2 gene was only detected in two *E. coli O157* isolates (İnat and Siriken, 2013).

Microbiological properties of 25 chicken breast and 25 thigh sold in Tokat were tested. According to the results; *E. coli O157*:*H7* were isolated form 6 (24%) chicken breast and 6 (24%) chicken thigh samples (Yıldırım et al., 2015).

30 samples, each of ready-to-cook meatballs and white cheese as well as 96 samples of various ready-to-eat foods (paste with hot pepper, American salad, cold white beans vinaigrette (barbunya pilaki), kisir, fried eggplant, fava, haydari, humus, sarma, mushroom salad, pasa mezesi, vegetable salad with yogurt, tarator) were collected from different outlets in Istanbul, and were investigated for the presence of verotoxins (consequently verotoxigenic *E. coli*) with immunoassay. Verotoxins were not detected in any of the tested samples (Bostan et al., 2005).

Examples of studies in the Middle East countries; during a period from March 2010 to March 2011, prevalence of *E. coli O157:H7/NM* in raw meat samples (beef, camel, sheep, goat, and water buffalo meat) was studied in Iran, and *E. coli O157* was found in beef samples (8.2%), water buffalo (5.3%), sheep (4.8%), camel (2.0%), and goat meat (1.7%). One out of 14 samples was determined to be serotype *O157*:*H7* and the others were determined as serotype *O157*:NM. Also *stx1, stx2, eaeA* and *ehlyA* genes were detected in 4 strains. It is demonstrated that the prevalence of *E. coli O157* occurred at the highest level in the summer (9.3%). It is emerged that in Iran, the beef and water buffalo meat are a significant source for *E. coli O157:H7/NM* infection (Rahimi et al., 2012).

The results of our study showed that prevalence of *E. coli O157* increased in April and May. In January, February and March, the samples taken from the 46 companies were found as negative for *E. coli O 157*. These companies such as caterings, restaurants were periodically checked for food production.

It was determined that the requirements of HACCP rules, hygiene criteria of preservation and storage conditions for foods must be fulfilled for eliminating *E. coli O157* and other pathogens with regular analysis carried out by authorized and accredited laboratories.

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