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# Determination of E. coli O157 in raw and cooked Doner kebabs by using IMS technique

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Summary

**Escherichia coli O157 is one of the major threats to public health due to the consumption of meat and meat products. The microorganism is known as a food-borne pathogen even in the presence of low levels. Therefore, a more sensitive method is required to be used for the detection of the microorganism. Immunomagnetic separation (IMS) is a technique used to improve the sensitivity of the detection. IMS has been used to determine the presence of E. coli O157 in raw and cooked Doner samples in this study. 3 out of 30 raw Doner samples have been found to be contaminated with E. coli O157. None of the cooked samples were carrying the agent.**

**Keywords: IMS, Doner kebab, E. coli O157**

Since Verotoxigenic *Escherichia coli* (VTEC) has been identified as causative agent of bloody diarrhea in 1982 (19), many studies have aimed to define the prevalence of this bacteria in food and foodstuffs. Because of its pathogenic and even lethal effects in some cases, concerns about *Escherichia coli* (*E. coli*) O157 infections increase constantly (3, 19). At the onset of the disease, symptoms of hemorrhagic diarrhea, Thrombotic Trombocytopenic Purpura (TTP), Hemolytic Uremic Syndrome (HUS) are generally recognized and usually results in death (19, 23, 24). 55 out of 169 residents and 18 out of 137 staff members have been affected at a nursing home during the outbreak that occurred in September 1985. HUS had developed in 12 patients and 11 of these 12 patients have (% 91,7) died (9). Another outbreak have occurred in North Dakota in 28-29 July 1990 (4). It was determined that this outbreak which affected 70 people was due to consumption of roast beef.

Meat and meat products are the major source for *E. coli* O157 infection. There are several outbreaks that have been reported involving beef consumption (4, 9). However, The Food and Drug Administration (FDA) recently issued an alert about an outbreak relating to the consumption of fresh spinach. This information has proved that outbreaks are not only limited to the consumption of beef products but to leafy vegetables as well (7).

Small number of microorganisms (less than 100 cells/g) is also capable of causing infection. Thus, the detection of small number of *E. coli* O157 in the foodstuff is getting more important (15, 19). Studies on developing a method for determining single cell organisms showed that Immunomagnetic Separation (IMS) technique has a significant advantage when compared with the conventional culture methods. It is a commonly used method for rapid detection of *E. coli* O157 in foods, as indicated in FDA/BAM (13). The technique for the IMS involves in combining super paramagnetic polystyrene particles (beads) coated with a mixture of monoclonal and polyclonal antibodies against a common structural antigen of targeted bacteria. Beads are extracted by using a magnet, and incubated with live bacteria cells to provide binding of the said bodies (4). Results of several studies have shown that the application of IMS following the enrichment process with a convenient buffer was able to lower the detection limit for *E. coli* O 157 (12, 20, 22).

Doner kebab (Doner) is a traditional dish of meat originating from Anatolia. It has become very popular throughout the world in the recent years. Generally lamb or beef meet is used for making doner kebab but recently poultry meat is also used. Doner is produced by mixing red meat with spices and herbs, shaping it conically around a mill and slicing the outer part in thin layers when it is fully cooked against a horizontal flame. A certain amount of doner meat is wrapped in pita bread and served with fries and some vegetables (1, 17).

Several researchers have studied on determination of microbiological quality of Doner (1, 16-18, 25). It has been reported in the study of Acar and Ciftcioglu (1) that it was possible to determine *Salmonella* spp. and coliforms in cooked Doner samples collected from several fast food courts. The study of Ulukanli et al. (25) has indicated that 26.25% of Doner samples collected from

Kars (East Turkey) had positive biochemical results for *E. coli* O157:H7. 40% of the cooked Doner samples collected from Tekirdag (North-west Turkey, close to Istanbul) were found to be contaminated with *Salmonella* spp. and *Clostridium perfringens* (16). Samples collected from the city center of Erzurum were determined to be contaminated in 18.75% level by *L. monocytogenes* (18). *Enterobacteriaceae* is an indicative flora, which gives an idea about the hygiene status of Doner (14, 17). Therefore, *Enterobacteriaceae* content of the samples were analyzed for the evaluation of the hygiene status of the samples in this study. Although several studies were conducted to determine the microbiological quality of Doner, a limited number of researches have been carried out on the occurrence of *E. coli* O157 in Doner as expected in low numbers.

The aim of this study is to determine the presence of *E. coli* O157 in raw and cooked Doner samples collected from Istanbul by using the advantages of the IMS method.

## Material and method

30 raw and 30 cooked and sliced 100 g portions of Doner samples were collected from local cafe and restaurants in Istanbul. The samples were kept in cold chain until they were analyzed. Method for the enumeration of *Enterobacteriaceae* was conducted in all 60 samples by means of pour plate technique on VRBD agar and incubation at 37°C for 24-48 hours fallowing serial dilutions in 0.85% saline solution.

Enrichment procedure was applied for *E. coli* O157 analyses. For this purpose, 25 g of the sample is added into 225 ml Buffered peptone water contained in a sterile bottle and incubated at 37°C for 6 hours. Then, the magnetic plate of MPC-M (Magnetic Particle Concentrator-Type M, 120.09) (Dynal Biotech ASA, Oslo, Norway) was removed and necessary number of 1.5 ml Eppendorf tubes were loaded into the Dynal MPC-M. Dynabeads anti-*E. coli* O 157 (Dynabeads anti-*E. coli* O 157; Dynal ASA) were re-suspended until the pellet in the bottom disappears by using a vortex machine. 20 µl of Dynabeads was pipetted and dispensed into each tube. 1 ml of the pre-enriched filtered sample aliquot for immunomagnetic separation was added. Dynal MPC-M rack was inverted five times and incubated at room temperature for 10 minutes with gentle continuous agitation to prevent the beads from settling (Janke-Kunkel, Germany). Magnetic plate was inserted into the Dynal MPC-M and left 3 minutes for proper recovery of beads. The supernatant was removed and 1 ml PBS (Oxoid BR 14a) (pH 7.4) containing 0.05% Tween 20 (Merck, 8.22184) was added and this procedure was repeated for two more times. The aliquots (the dynabeads-bacteria complex and 100 µl PBS-Tween buffer) prepared by IMS technique were spread on SMAC--CT Agar (Sorbitol Mac Concey agar supplemented with 2,5 mg/l potassium tellurite and 0.05 mg/l cefixime) (Dynal® 740.01) plates in duplicate and incubated at 37°C for 18 hours. The non sorbitol fermenting colonies were detected for $\beta$-glucronidase activity on VRB-MUG (Oxoid® CM 978) agar and indol reaction and latex agglutination (Oxoid® DR 620). Agglutination positive in Latex test, indol positive and MUG negative colonies were identified as *E. coli* O157.

## Results and discussion

Doner kebab is one of the most popular fast-food in Turkey and in some other countries. Consequently, numerous studies have been conducted to evaluate the safety of this product (1, 14, 16-18, 25).

In this study, *Enterobacteriaceae* was selected as an indicator flora for the determination of the microbiological quality level. Members of *Enterobacteriaceae*, like *Salmonella* spp. and *E. coli* as well as Gram-positives such as *Staphylococcus aureus* and *Clostridium perfringens*, may be found in considerable numbers in Doner kebabs and could be an important risk factor for public health (14). *Enterobacteriaceae* counts for 30 raw and 30 cooked doner samples were given in tab. 1. According to the results of *Enterobacteriaceae* analysis, 2 out of 30 cooked samples (6.7%) and 27 out of 30 raw samples (90.0%) have been found to be in an unsatisfactory condition with respect to the category 3 of the PHLS microbiological guidelines for some ready to eat foods, which includes sliced meat (beef, poultry etc.) (11).

It has been reported that overgrowth of competitive flora, especially coliforms, could occur even in the usage of selective enrichment mediums. Noveir et al. (21) have isolated some non-selective bacteria, which were mainly *Enterobacteriaceae* members, in selective mediums used in their research. Therefore, the use of IMS technique would be very supportive to eliminate the competitive flora and selective growth before the inoculation on agar plates. Because, this competitive flora might mask *E. coli* O157 colonies on agar plates (21). Our previous research has proved that more accurate detection and isolation have been obtained by using IMS technique (22). Other researchers have also reported that the more precise results have been obtained by using IMS technique before the isolation process on a selective medium (12, 20).

*E. coli* O157:H7 has been introduced as one of the most important pathogen for food borne outbreaks since it was first identified in 1982 (19). *E. coli* O157:H7 outbreaks in 1993 were generally reported as food-borne diseases due to the consumption of undercooked ground beef by CDC (5). Similarly, the results of several other researchers indicated that *E. coli* O157 was an important risk factor associated with ground beef and preparation

**Tab. 1. Distribution of raw and cooked Doner samples regarding *Enterobacteriaceae* counts divided in 1 log scale and their evaluation for microbiological quality according to the category 3, PHLS microbiological guidelines**

| | Log cfu/g | | | | | | |
|---|---|---|---|---|---|---|---|
| | < 1 | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 | > 6 |
| Raw Doner Samples | 0 | 0 | 0 | 3 (10%) | 5 (16.7%) | 21 (70.0%) | 1 (3.3%) |
| PHLS, Category 3 | Satisfactory 0 (0.0%) | | Acceptable 3 (10.0%) | | Unsatisfactory 27 (90.0%) | | |
| Cooked Doner Samples | 12 (40.0%) | 5 (16.7%) | 7 (23.3%) | 4 (13.3%) | 2 (6.7%) | 0 | 0 |
| PHLS, Category 3 | Satisfactory 17 (56.7%) | | Acceptable 11 (36.7%) | | Unsatisfactory 2 (6.7%) | | |

methods as well (2, 3, 21). The results of the study by Alisarli and Akman (3) suggested that 4.66% of the ground meat samples collected from Van were found to be contaminated by *E. coli* O157. Similarly, Aksu et al. (2) determined that 6% of the minced meat samples and 2% of the meat ball samples harbored *E. coli* O157. In another study, 26 (25%) of the Doner samples collected from local restaurants were positive in both serological and biochemical tests performed for the identification of *E. coli* O157:H7 (25).

*E. coli* O157 has been identified in 3 out of 30 raw Doner samples (10.0%) and has not been identified in 30 cooked Doner samples in this study. These 3 *E. coli* O157 positive samples were among the samples with unsatisfactory conditions in terms of *Enterobacteriaceae* counts. These results were different than the findings of Ulukanli et al. (25), who determined that 26.5% of the samples were positive for *E. coli* O157. This might be due to sufficient heating process applied to the Doner in the region where the study was conducted. Similar to the *E. coli* O157 results, *Enterobacteriaceae* numbers in the cooked samples were significantly low (in the level of 2 log) when compared with that of the raw samples. These results were similar to that of Kayisoglu et al. (16), who suggested that coliform counts in the cooked samples were 2 log lower than raw samples. *Enterobacteriaceae* counts were also below the detection limit (< 1 log cfu/g) in 40% of the cooked samples. Those results have indicated that cooking process was effective on the viability of *Enterobacteriaceae* in Doner.

However, one should bear in mind that the study was conducted with Doner meat. Doner sandwiches might still be risky due to cross contamination or un-sufficient quality of salads and dressing served with Doner. A *Salmonella typhimurium* outbreak associated with kebabs occurred in South Wales (July 1995) supports this theory. Yogurt, which is served with kebab as a side dish, was found to be reason for the outbreak. Further investigations involving the outbreak have shown that the uncovered yogurt containers were being stored under raw meat rack in the cooler (10). Although the studies have concentrated on the presence of *E. coli* O157 in beef and products, the risk of the microorganism in fresh green vegetables has been proved by later experiences. The latest outbreak informed by FDA in September 2006 was related to the consumption of raw spinach. The outbreak affected 183 people form 26 different states of the country (7).

The presence of *E. coli* O157 in many foods has been proved by various research activities. Nevertheless, not many *E. coli* O157 outbreaks have been reported so far. This should be the reason of the weakness on detecting or reporting of the food-borne outbreaks all over the world. Supportively, it is mentioned in the conference report of FAO/WHO PAN-European Conference On Food Safety And Quality that; only 1-10% of cases comes to the knowledge of the official agencies and the extent of under--reporting varies from country to country (6).

In conclusion, the destruction effect of heat process was found to be adequate for the inhibition of *E. coli* O157 in Doner samples which were analyzed. However, the risk of cross contamination and the risk from the side materials served with Doner still exist. Therefore, strict compliance with the rules of hygienic aspects, such as HACCP and GMP programs, at all stages from manufacture to consumption of Doner kebabs is essential. Observance of these rules, especially for the Doner served in fast food restaurants, would support sufficient product quality and minimize the risks resulting from the contamination of *E. coli* O157, as well as other pathogenic microorganisms.

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