

Detection of *Escherichia Coli* O157:H7 from Beef Doner Kebabs Sold in Kars

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ABSTRACT

In this study, a total of 80 cooked doner samples collected from various restaurants in Kars during May and June, 2004 were analyzed for the presence of *Escherichia coli* O157:H7. Samples were pre-enriched in modified EC broth+novobiocin (mEC+n). Sorbitol MacConkey Agar, supplemented with 0.05 mg Cefixime and 2.5 mg Tellurite (CT-SMAC agar) and Violet Red Bile + MUG Agar media were used in the isolation of *E. coli* O157:H7 colonies. Presumptive colonies were subjected to biochemical tests and confirmed by using *E. coli* O157 latex test kit. Out of 80 doner samples, thirty seven doner samples (46.25%) gave positive results for the presumptive *E. coli* O157 colonies. Out of these, only twenty-one (26.25%) samples positive for *E. coli* O157 complied well with the biochemical tests. Seven (8.75%) isolates positive for *E. coli* O157 could not be confirmed by the biochemical tests. Four of these were identified as *Citrobacter freundii* and remaining three as *Hafnia alvei*. Using H7 antiserum, only nine (11.25%) of the 37 doner isolates were found to be positive for *E. coli* O157:H7. The occurrence of *E. coli* O157:H7 resotype in cooked doners indicates that there may be a potential risk for public health from inadequately cooked and/or recontaminated cooked doner kebabs.

Key Words: E. coli O157:H7, beef meat, doner kebab, public health.

1. INTRODUCTION

E. coli is one of the bacteria that exists in the normal microflora of the intestinal tract of humans and warmblooded animals. Most strains of E. coli are nonpathogenic [1], however some strains differ from commensals in that they express virulence factorsmolecules directly involved in pathogenesis thereby causing disease [2]. Shiga-toxin producing E. coli strains (STEC) causing human infections belong to a large number of O:H serotypes are also named as verotoxinproducing E. coli (VTEC). STEC strain of E. coli O157:H7 is a food borne pathogen being the most common enterohaemorrhagic (EHEC) serotype and has been associated with a variety of diseases in humans including diarrhoea, haemorrhagic colitis and the haemolytic uraemic syndrome [3-5]. Cattle have been regarded as a natural reservoir of VTEC organisms for infections [6,7]. Most human infections with E. coli O157:H7 have been primarily associated with the

consumption of contaminated and improperly cooked ground beef and unpasteurised cow's milk [8,9] and many food borne outbreaks of *E. coli* O157:H7 have been reported in different countries [10-14]. In Turkey, some work has been published regarding *E. coli* O157:H7 in minced beef, soudjouk, hamburger, cooked meat ball and raw meat ball samples [15-21].

Doner kebab is a popular meat product consumed widely in Turkey and in many countries around the world [22-25]. To our knowledge, the microbiological quality of the doner kebabs has been a research issue for the last two decades but detection of *E. coli* O157:H7 particularly in cooked beef doner kebabs has not been studied previously in Turkey. Furthermore, in previous studies, seasonal occurrence of enterohaemorrhagic (EHEC) isolates and sporadic or outbreaks caused by these pathogens revealed that increased recovery from various materials were mostly detected in warm seasons such as summer [26,27]. Therefore, this study aimed at investigating E. coli O157:H7 in cooked doner meat for whether it poses a risk for public health and it may also be effective to find out the incidence of these bacteria in cooked doner meat considering the sampling months as being surveyed between May and June.

2. MATERIALS AND METHODS

A total of 80 samples of sliced cooked beef doner kebabs (100 g each) on the serving were collected randomly from different 20 restaurants in Kars. Samples were collected weekly between mid. May to mid. June, 2004. Samples were transported to the laboratory in a cooling box and processed immediately upon arrival. The sampling time chosen was during the lunch times which were the busiest hours for serving.

A 25 g of doner sample was homogenized with 225 ml of modified EC broth containing 20 mg/l novobiocin (mEC+n)(mEC+n/1.14582, Merck, Germany) and preenriched for a period of 24 h at 37 °C. Then, incubated broth culture was streaked onto Sorbitol MacConkey Agar (CT-SMAC Agar/109207, Merck, Germany) containing 0.05 mg/l cefixime and 2.5 mg/l tellurite. They were then incubated at 42 °C for 24 h. After incubation, the plates were checked for the presence of sorbitol negative, 1-2 diameter colorless colonies. Subsequently, these presumptive colonies were re-streaked onto Violet Red Bile (1.01406, Merck, Germany) + 4-methylumbelliferylβ-D-glucuronide (MUG) (Oxoid BR071E) Agar (42 °C/24-48h). After incubation, the latex agglutination test was performed to the pink colonies showing no fluorescence in order to confirm presumptive colonies and to determine the serotype of the bacteria using specific the E. coli O157 test kit (Oxoid, UK) and H7 antisera (Seiken Denka Seiken Co., Ltd., Tokyo) as described by the manufacturers. Agglutination positive isolates were also subjected to the biochemical tests since false positive results have been observed due to cross reactions with the

other sorbitol negative strains (such as E. hermanii) and microorganisms (such as Salmonella. Shigella. Providencia and Citrobacter, particularly C. freundii) [28-30]. The biochemical tests included Gram staining, catalase, ONPG test, arginine, ornithine and lysine decarboxylase tests, hydrogen sulphide production, urease test, tryptophan, indole, methyl-red test, voges-proscauer test, citrate utilization, gelatin liquefaction, nitrate reduction test, O/F tests, fermentation tests including glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, arabinose and amygdaline tests. Additionally, potassium cyanide test was also used for all isolates [31].

3. RESULTS AND DISCUSSION

This study has focused on cooked doner kebabs. Out of 80 samples, thirty-seven (46.25%) samples gave positive results for E. coli O157:H7 with the O157 antiserum. All of these O157 antiserum positive results, however, could not be confirmed by the biochemical tests carried out. Only twenty-one (26.25%) samples positive for E. coli O157 using O157 antisera were complied well with the biochemical tests (Table 1) whereas, seven (8.75%) isolates found positive for E. coli O157 with O157 antiserum test could not be confirmed by the biochemical tests.

Considering the biochemical tests, four strains were identified as Citrobacter freundii while three strains were identified as Hafnia alvei. Although selective enrichment and plating is one of the widely accepted techniques, cross-reactions of antiserum O157 and H7 with other microorganisms were also reported in previous studies [32,33]. Thus, these seven O157 antiserum positive results for E. coli O157 may be the result of cross-reactions and are in accordance with the previous findings of Silveira et al. [32] and Noveir et al. [33] in minced meat, hamburgers and soudjouk samples, respectively. H7 antiserum was also applied on to all O157 positive isolates using the slide agglutination. Only nine (11.25%) of the 80 doner samples were found to be positive for E. coli O157:H7 (Table 1).

0157 (+) 0157 (+) C. freundii-H. alvei Sample No H7 (+) (Serologically) (Biochemically) (Biochemically) (Serologically) 80 (100%) 37 (46.25%) 21 (26.25%) 4 (5%) - 3 (3.75%) 9 (11.25%)

Table 1: Identification of presumptive colonies serologically and biochemically.

In developed countries, the incidence of E. coli O157 and/or E. coli O157:H7 in food commodities and outbreaks due to E. coli O157:H7 are well documented [34-41]. These studies indicate that in particular, ground beef is the main agent responsible for outbreaks in the USA

and some European countries with an occurrence varying from 0 to 3.7% in food [42].

Higher levels (4.8%, 8.7%) of E. coli O157 have also been reported in developing countries [30, 43-45].

In Turkey, studies conducted to detect E. coli O157 and/or E. coli O157:H7 revealed that E. coli O157 have been isolated from meat and meat products with an occurrence varying from 0 to 5% in meat and meat derived products such as hamburgers and meat balls [15-17]. Few studies, however on the isolation of E. coli O157:H7 from ground beef and beef products in Turkey have revealed negative results [15,16,46,47]. Likewise, there is also no report of any outbreak due to E. coli O157:H7 in Turkey [20]. It is likely that these kinds of cases have not been reported or the cause agents of food poisonings have not been identified. Thus, it is also not possible to compare directly the results of this study with previously published studies because of the use of a variety of different food materials. But it seems that incidences found in this study seems to be higher than those previous studies for other food products.

The microbiological quality of the doner kebabs have indicated that doners in retail stores may have a high potential risk of food borne diseases for the consumers [22, 48-52]. Among these, the presence of E. coli in both raw or cooked doners is a matter of particular concern. Level of E. coli in either raw or cooked doners or in both cases appears to be very high [20,22,24,25]. Although high or low levels have been reported in those studies, to our knowledge, this is the first to report the isolation of E. coli O157:H7 particularly from cooked doners in Turkey. Although gene encoding the virulence factors have not been detected in this work, obviously, the presence of E. coli O157:H7 in cooked doner samples may pose a potential risk of hazard for the public health. Such high incidences of contamination as we found are most likely due to insufficient heat exposed to the doner cone. The comminution of whole raw meat to produce such products as burgers, doners etc., may distribute the initial surface contaminants throughout the derived product where they may be protected during subsequent heating processes. Although VTEC has not been shown to be more heat resistant than other E. coli, variations in observed heat resistance can however occur due to a number of product specific factors. For example, the presence of fats may increase thermal tolerance, as will high levels of a competitive microflora. Both these factors may relate to the reduction in the rate of heat transfer through the product and/or a reduction in water activity. Cooked meat products can also support the survival of VTEC and act as a transmission vehicle for the food poisoning pathogens, since a number of food-poisoning outbreaks have been associated with recontamination of such products. The persistence of VTEC on equipment and surfaces may be related to such incidents since VTEC E. coli O157 has been shown to survive for extended periods on stainless surfaces and can grow on plastic cutting boards in the presence of meat juices [53].

In this work, contamination vehicles such as the quality of the raw meat, personal hygiene in the kitchen and kitchen utensils, various ingredients used in the preparation of doner meat such as milk, spices, herbs, vegetables (onion, tomato) have not been investigated but vegetables, milk and water have also been implicated in *E. coli* O157:H7 poisoning outbreaks [54-56]. Therefore health hazards associated with these factors must be analyzed and the consumers should be informed regarding the quality of the food to protect their health from the contamination and/or cross contamination via the improperly cooked meat type products.

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