

The Occurrence of *Escherichia coli* O157:H7 in the Ground Beef and Chicken Drumsticks.

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SUMMARY

Escherichia coli O157:H7, predominantly originated from beef, is a significant pathogen to the public health and thus, needs to be vigorously surveyed in meat samples. Three (6%) of 50 ground beef samples were found to be contaminated with *E. coli* O157:H7, but it was not detected any of 50 chicken drumsticks. This contamination rate of ground beef samples indicated that unhygienic practices prevailed in slaughterhouses in Kars-Turkey. To our knowledge, this is the first to report the isolation of *E. coli* O157:H7 from ground beef in Turkey.

INTRODUCTION

Escherichia coli O157:H7 is a food-borne pathogen, primarily associated with the consumption of contaminated ground beef and is an important food safety concern worldwide (9). One of the common modes of transmission is accepted to be the consumption of contaminated beef and beef products. Additionally, ground beef is the most studied beef product for the presence of *E. coli* O157:H7 in Turkey and other countries. Developed countries have implemented strategies to eradicate the agent from livestock for the protection of public health (14). According to Turkish Food Legislation, every 25 g of fresh beef and fresh beef, products including ground beef, marketed in Turkey since 2000 should be free of *E. coli* O157:H7 (12). To date no infection of human or animal by *E. coli* O157:H7 has been reported, but the presence of this agent in human fecal samples was first stated in 1995 (18) and in cattle fecal samples in 1996 (1). Positive isolation of *E. coli* O157 from ground beef and hamburger samples was reported in Turkey in 1998 (5,13). Contamination of chicken carcasses has been reported abroad (3), but it has not yet been researched in Turkey. In this study, we therefore investigated whether *E. coli* O157:H7 is present in ground beef and chicken meat marketed in Kars city; Turkey.

MATERIALS and METHODS

Test strains. *E. coli* O157:H7 (strain no. 937) was kindly provided by Dr. Y. Ozbas (Univ. of Hacettepe, Ankara-Turkey) and *E. coli* strain EC1 isolated from ground beef in our laboratory was used as the reference strains. Each strain was maintained at 4°C on tryptic soy agar (Difco Laboratories, Detroit, Mich.) supplemented with 0.6% yeast extract (TSA-YE) (Difco) slants with monthly transfer.

Food samples. A total of 50 ground beef and 50 chicken drumstick samples (500 g each) were obtained from various local retail outlets in Kars city;

Turkey. The samples were delivered to the laboratory within 2 h in portable refrigerator and were examined for the presence of *E. coli* O157:H7. Before analysis the chicken drumsticks were maintained at 4°C for 6 hours in order to defrost them. Then, each lot of sample was mixed in a sterile bag by hand massaging. A 25 g portion of ground beef or one drumstick from each lots of samples were transferred to separate sterile bags. These sub-samples were hand massaged and the bags were incubated at 25°C for 2 h with the addition of 250 ml mEC broth in order to resuscitate naturally injured cells. Then, this enrichments were supplemented with novobiocin (20 mg/litre) (n, Merck, Darmstadt, Germany) Following supplementation, the samples were incubated at 42 °C for 22 h. After incubation, tenfold serial dilutions of the enrichment cultures were made using 9 ml of 0.85% saline solution. Fifty $\frac{1}{10}$ portions from the fourth to sixth dilution tubes were each streaked paralelly separately onto sorbitol MacConkey medium (SMAC) and *E. coli* O157:H7 medium (EOH). After an incubation period of 18-24 h, ten typical whitish gray colonies on SMAC, and typical pink colonies on EOH were picked and analysed for the presence of *E. coli* O157:H7 (6,8).

Selected colonies were picked from SMAC and EOH and streaked separately onto SMAC supplemented with cefixime-tellurite (CT, Difco) (CT-SMAC) to investigate CT resistance and the purity of the colonies. All the selective medium plates used in this study were incubated at 42°C for 18-24 h. After the incubation period, the typical CT resistant colonies were picked and tested for the utility of sorbitol and methyumbelliferyl- β -

glucuronide in modified Haemorrhagic Coli (mHC, Difco) broth.

All the methods used in this study for the further identification of *E. coli* O157 are described by the FDA- Bacteriological Analytical Manual (6). Briefly, suspect colonies were tested for the ability to ferment lactose and sucrose in Triple Sugar Iron agar (TSI, Difco) slants, indol production, methyl red and Voges Proscauer reactions, citrate utilisation (the IMVIC tests) and typical colony morphology on Levine Eosine Methylene Blue agar (L-EMB, Difco). Presence of the O157 antigen was investigated by the latex agglutination test using the *E. coli* O157 test kit (Oxoid). Antisera contained in a commercially available O:H serotyping kit (*Escherichia coli* antisera (SEIKEN, Denka Seiken Co., Ltd., Tokyo, Japan) was utilised for O:H serotyping following the manufacturer's specification.

RESULTS and DISCUSSION

We detected only 7 strains of *E. coli* O157:H7 from three (6%) out of 50 ground beef samples. However, it must be taken into consideration that the cultural methods used in this study may contribute to the low isolation rate. The exact contamination rate may be at least two times higher than stated here due to the low isolation rate of culture methods compared to other immunological and genetical methods and also in the light of high diversity in the success of the enrichment and plating media used in studies (2,11). Considerably higher isolation rates of *E. coli* O157:H7 than in this study have been reported elsewhere. In south Africa it was isolated from a total of 74.5% and in Malaysia from 36% of beef samples (11,17). On the other hand, in some studies beef and beef samples have found to be free (14,16) or to yielded lower contaminated sample rates (7,10,15) than those detected in this study. Dutta et al. (4) isolated *E. coli* O157:H7 from 2 (9%) out of 22 minced beef samples in India. Doyle and Schoeni (3) has isolated *E. coli* O157:H7 from 4 (1.5%) of 263 poultry samples and stated that the organism is not a rare contaminant of poultry meats. But, in this study we have not isolated the organism from chicken drumsticks.

Studies on *E. coli* O157:H7 isolation from ground beef and beef products in Turkey have yielded negative sample results (1,5,13). However *E. coli* O157 has been isolated in Turkey (5,13) from other foods. To our knowledge, this is the first to report the isolation of *E. coli* O157:H7 from ground beef in Turkey.

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