PREVALENCE OF VEROCYTOTOXIGENIC ESCHERICHIA COLI O157:H7 ON CHICKEN CARCASSES SOLD IN TURKEY

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Abstract

The prevalence of Escherichia coli O157:H7 was investigated on samples of various portions of 190 chicken carcasses, obtained from local retail markets and poultry shops in Turkey. Immunomagnetic separation methods was used to isolate E. coli O157:H7 from the samples. Verotoxin 1 (VT1) and verotoxin 2 (VT2) productions by the isolated E. coli strains was also investigated. E. coli O157:H7 was isolated from two (1.05%) of the 190 samples of poultry meat examined. All the strains isolated produced both VT1 and VT2. These findings indicate that poultry meat can also be a source of E. coli O157:H7 infections for humans. Appropriate control measures should be developed and implemented to eliminate this human pathogen from foods of animal origin, including poultry, in order to protect human health.

Key words: poultry, chicken carcasses, immunomagnetic separation, verocytotoxigenic E. coli O157:H7.

Among six classes of diarrhoeagenic Escherichia coli, enterohaemorrhagic E. coli (EHEC) is recognized as an important agent of bloody, and none bloody diarrhoea in humans. Two toxins referred to as verotoxin 1 (VT1) and verotoxin 2 (VT2) or shiga toxins (Stx1 and Stx2), are the main determinants of the disease caused by EHEC strains (25). These toxins are produced by several serotypes of E. coli, but bloody diarrhoeal diseases are mostly caused by E. coli O157:H7, which is one of the EHEC groups (23, 29). In addition to haemorrhagic colitis, this serotype is also recognized as the cause of diarrhoea-associated forms of haemolytic uraemic syndrome, and thrombotic thrombocytopenic purpura in humans. Since the first isolation of E. coli O157:H7 from an outbreak of human bloody diarrhoea in 1982, it has been reported from hundreds of sporadic cases and outbreaks in more than thirty countries throughout the world (9).

Due to low infectious dose and life-threatening complications of E. coli O157:H7, the organism has emerged as an important zoonotic agent, causing serious public health problems. Most E. coli O157:H7 outbreaks are food or water-related (17, 18). In particular, undercooked ground–beef has been considered to be the main cause of several outbreaks (23), but also other foods of animal origin, including meat products, milk, and milk products such as cheese and yoghurt, and raw vegetables, apple juice, and cider have all been implicated as sources of some outbreaks (3, 5, 19). Human infections have also been associated with direct contact with animals (21, 22).

E. coli O157 can normally be found in the gastrointestinal system of a range of domestic animals including cattle, sheep, and poultry, of which cattle are known to be the major reservoir for human infection (11). It has also been isolated from a variety of foods such as beef, lamb, pork, and milk and their products (10, 12, 13, 29).

Immunomagnetic separation (IMS) techniques, used in conjunction with a selective agar plate such as sorbitol MacConkey agar (sMAC) or most common cefixime - potassium telluride - sMAC, allowed the isolation of E. coli O157 from various type(s) of samples (12, 29). IMS techniques have been reported to be faster and markedly more sensitive than conventional methods, in the isolation and identification of E. coli O157 strains (29).

The present study was undertaken to determine the prevalence of E. coli O157:H7 on various portions of chicken carcasses, obtained from retail markets and poultry shops in Turkey, by means of IMS technique. Additionally, VT1 and VT2 production by the isolates was determined.
Material and Methods

Sample collection. A total of 190 fresh chicken carcass samples comprising of drumsticks (n=65), skinless breasts (n=60), and wings (60 breast meat), purchased from local retail markets and poultry shops in Afyon, Turkey, were examined. The samples were obtained between April and July 2004, brought to the laboratory in ice, and examined immediately on the day of purchase.

Isolation and identification of E. coli O157:H7. Twenty-five grams of each sample were placed into stomacher bags, and 225 ml of sterile buffered peptone water (BPW) (Oxoid-CM 509) containing novobiocine (20 mg L⁻¹) was added and homogenized for about 2 min using a stomacher (Bagmixer, Interscience) and incubated aerobically for 18 h at 37°C.

Dynabeads consisting of magnetic beads coated with E. coli O157 antibody (Dynabeads® anti E. coli O157, Dynal, Norway) were used for the IMS technique. Briefly, 20 µl of the Dynabeads solution was mixed with 1 ml of the enriched culture in an Eppendorf tube and incubated for ca 10 min at room temperature with continuous agitation, according to the manufacturer’s instructions. Phosphate-buffered-saline (PBS)-Tween was used as washing diluent, and the washing step was repeated twice. Finally, the beads were re-suspended in 100 µl of the washing diluent by brief vortexing. A volume of 50 µl of re-suspended Dynabeads was inoculated onto cefixime - potassium telluride -sMAC (Oxoid, CM813B), which consisted of sMAC, and supplemented with cefixime (50 mg) and potassium telluride (25 mg) in duplicate. The plates were then incubated aerobically at 37°C for 24 h, as described earlier (12).

After the incubation, up to five suspected sorbitol negative colourless colonies from each agar plate was picked up and plated onto Violet Red Bile Agar (Oxoid, CM 107) containing 4-methylumbelliferyl B-D glucuronide (MUG, Oxoid, BR071E), and incubated aerobically for 24 h at 37°C for the detection of β-glucoronidase activity. The β-glucoronidase activity was examined under a UV-light (Camag, 4 W/366 nm, UK) in the dark, using the colonies grown on VRBL agar, and the colonies showing no fluorescence activity were accepted as β-glucoronidase negative. These colonies were examined with E. coli O157 latex agglutination test (Oxoid, DR623 M) and the isolates that were found to be positive with the slide agglutination test were further tested for biochemical identification using the API 20E (BioMerieux Vitek, Hazel Wood, USA). After that, cellobiose (Sigma, C7252) and H7 (Denca Seiken, 211057, Japan) tests were applied, and cellobiose negative - H7 positive isolates were identified as E. coli O157:H7 (16).

Detection of verotoxins. The production and type of verotoxin synthesised by the E. coli O157:H7 isolates were identified by Glisa Duopath Verotoxin test (Merck, 1.04144.0001). Briefly, each E. coli O157:H7 isolate was inoculated into CAYE Broth + Carbodox, and the broth was incubated aerobically for 6 h at 37°C. Afterwards, 180 µl of CAYE culture were taken into a tube supplemented with polymyxin, and incubated for further 10 min at 35-37°C. A volume of 160 µl of this culture was then transferred to a test device, in order to detect the production and type of verotoxin (20).

E. coli O157:H7 963 strain, kindly provided by Prof. M. P. Doyle (Center for Food Safety Quality Enrichment, University of Georgia Griffin, Georgia, USA), was used as positive control.

Results and Discussion

E. coli O157:H7 was isolated from 2 of the 190 samples (1.05%) examined. Both isolates were found to be capable of synthesising VT1 and VT2.

It was reported that E. coli O157:H7 could readily colonise the caeca of chickens, and excreted in chicken faeces for several months (4, 28). Schoeni and Doyle (26) also found that a day old chicks infected with E. coli O157:H7 strains can shed these microorganisms for more than 11 months, suggesting that chickens may serve as a potential reservoir for this human pathogen. They were also able to isolate this bacterium from the eggshells surface of the hens that were faecal shedders.

A number of studies have been carried out to determine the prevalence of E. coli O157:H7 in poultry. Doyle and Shoeni (15) assayed 263 fresh, uncooked complete chicken samples, and isolated the organism from 4 (1.5%) samples. In Thailand, Suthienkul et al. (27) found 9 (1%) out of 107 chicken carcasses contaminated with E. coli that did not produce Shiga-like toxin. In another study performed in the UK, 1-2% of chicken, lamb and pork meat samples, were found to be contaminated by E. coli O157:H7 (2). In Egypt, Abdul-Raouf et al. (1) examined 50 boneless chicken meat samples, and were able to detect E. coli O157:H7 in 2 samples. Vernozy-Rozand et al. (29) analysed 250 samples of meat and meat products in France, and found 4 chicken meat samples (1.6%) to be positive for non-verotoxin producing-E. coli O157. The results of the present study, in which 1.05% (2 positive out of 190 samples) contamination rate of E. coli O157:H7 was found, generally agrees with the results obtained in previous studies (1, 2, 27, 29). The reason for the presence of E. coli O157:H7 on chicken carcasses, may result either from cross-contamination during slaughter, and/or processing or during transportation. Cross-contamination of the carcasses at the chicken slaughterhouse can be at various stages such as, during evisceration, scalding, plucking, and/or cutting processes. The presence of E. coli O157:H7 on chicken carcasses suggests that chickens may be natural carriers of the microorganism, or contamination may be coming from other sources such as transportations, and/or water used during slaughtering for various purposes. E. coli O157:H7 is regarded to maintain its natural long life cycle in the environment by its resistance to cooling, freezing and to acidic conditions (7, 18).
This pathogenic type of *E. coli* draws attention, due to its potential to produce severe, life-threatening illness in humans, and its low minimal infective dose (8, 11). It causes a wide spectrum of clinical symptoms ranging from mild diarrhoea, to haemorrhagic colitis, and haemolytic-uraemic syndrome (HUS) in humans. Most outbreaks due to this bacterium are considered to be caused by foods of animal origin, including poultry meat, or water (24). Consumption of cold turkey meatballs was reported to be responsible for an outbreak of *E. coli* O157:H7 in the UK (2). It is noteworthy to state that both isolates of *E. coli* O157:H7 obtained from chicken meat in the present study, produced VT 1 and VT2. Verotoxin production by *E. coli* O157:H7 is considered to have an important role in the pathogenesis of haemorrhagic colitis and HUS.

In conclusion, the presence of *E. coli* O157:H7 in chicken meat determined in the present study, suggests that chicken may be a source of human infections due to this organism. In addition, verotoxin production of the both chicken isolates, stresses the importance of this source of *E. coli* O157:H7. In order to protect public health, a series of control strategies should be developed and implemented at all stages of food production, system “from farm to table”, to eliminate contamination with this important human pathogen. More epidemiological studies are needed in order to determine the possible role of poultry as a source and/or reservoir of *E. coli* O157:H7. In addition, the exact mode of entry of this bacterium into food chain should be determined in order to develop and implement control measures.

References


