



ISOLATION OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN STOOLS OF PATIENTS WITH GASTROENTERITIS

Microbiology

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ABSTRACT

In many countries of the world, *Escherichia coli* O157:H7 has been detected as a causative agent in cases with gastroenteritis. There is limited number of studies conducted with *E. coli* O157:H7 in Turkey. In the present study, we investigated the bacterium in 200 patients with bloody stool as well as in 50 unpasteurized milk samples using ideal isolation techniques. Stool and milk samples were inoculated into the Sorbitol MacConkey Agar, which is selective for *E. coli* O157:H7 and in other mediums. *E. coli* strains unable to ferment sorbitol were exposed to agglutination using the anti-serum prepared against O antigen. *E. coli* O157:H7 was not detected either in 200 patients or 50 milk samples. Enterohemorrhagic *E. coli* serotype O157:H7 is not a common bacterium in Turkey. Exploration of this agent, which is found in other countries, should not be neglected particularly in patients with gastroenteritis with a history of bloody stool or eating suspicious foods.

KEYWORDS

Escherichia coli O157:H7, sorbitol, gastroenteritis

INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 is a bacterium considered responsible primarily for the etiology of hemorrhagic colitis as well as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). In clinical cases, it is isolated from the diarrheal stool samples with or without blood. In addition, the bacterium can be isolated also from rare bovine meat and unpasteurized milk samples. In the Northern America and Canada in particular, *E. coli* serotype O157:H7 has been isolated in the epidemics of hamburger-associated food intoxication (Kassenborg et al., 2004; Schaffner and Schaffner, 2007). *E. coli* O157:H7 produces Shiga-like toxin. Verotoxin-producing *E. coli* O157:H7 plays an important role in the pathogenesis (Mead & Griffin, 1998; Wells et al., 1991).

In Turkey, studies on EHEC infections, which have been usually reported from American and European countries in the recent years, are inadequate in number. Literature review has indicated limited number of studies conducted in gastroenteritis cases in Turkey (Hascelik, Akan, Diker, & Baykal, 1991). Studies from Turkey have revealed that cattle are significant reservoirs for verotoxigenic *E. coli* O157:H7 (Aslantaş, Erdoğan, Cantekin, Gülaçtı, & Evrendilek, 2006; Yılmaz, Gun, Ugur, Turan, & Yılmaz, 2006). In abroad, sporadic cases and outbreaks have been reported caused by Turkish-origin VTEC (Smith-Palmer, Locking, Reilly, & Fisher, 2005).

E. coli O157:H7 is a bacterium isolated quite rarely. The most significant character that differentiates *E. coli* O157:H7 from other *E. coli* strains is its inability to ferment sorbitol (March, & Ratnam, 1986). The present study was conducted to explore this bacterium in the stools of patients with gastroenteritis, primarily in patients with hemorrhagic colitis, and in unpasteurized milk samples, using the recommended ideal isolation techniques and to illuminate the situation in Turkey.

MATERIALS AND METHODS

Gastroenteritis patients' bloody stool samples, which was sent to Gazi University Medical Faculty Hospital, and fresh raw milk samples collected from various districts of Ankara province were examined. Isolation was performed in 200 stools and 50 milk samples. Sorbitol MacConkey Agar (SMAC, Oxoid, England), Eosin Methylene Blue Agar (EMB-Oxoid, England), blood agar, *Salmonella-Shigella* agar (SS-Oxoid, England), Selenite F Broth (Merck, Germany), Nutrient Broth (Merck, Germany), and Mueller-Hinton agar (Oxoid, England) were used for the stools of gastroenteritis patients. SMAC, EMB, blood agar, Löwenstein-Jensen Medium (Difco, Kansas, USA), Nutrient Broth, and Mueller-Hinton agar were used for unpasteurized milk samples. As the control strain, *E. coli* O157:H7 was obtained from the laboratory of Microbiology Department of Hacettepe University Medical Faculty Hospital. A loop full of stool sample taken particularly from the bloody, purulent and mucoid parts of the stools of gastroenteritis patients was mashed in 0.5 mL normal saline. A loop full of sample taken from this stool suspension was inoculated as a

single colony into the SMAC, EMB, and blood agar. The stool samples, which were inoculated into the Selenite-F Broth for 6-8 hours, were then passed into the SS agar. Unpasteurized milk samples collected from various environments were directly inoculated into the SMAC, EMB, Löwenstein-Jensen, and blood agar. Among the colonies that did not use sorbitol in SMAC, those determined to be consistent with *E. coli* by conventional methods were confirmed using *E. coli* O157 antiserum (Denka Seiken, Japan).

RESULTS

Leukocyte was detected via direct microscopic examination in 40% of the stool samples taken from 200 participants. Of these samples, EIEC was positive in 9 (4.5%), *Klebsiella spp.* was positive in 7 (3.5%), EPEC was positive in 3 (1.5%), *Salmonella spp.* was positive in 2 (1%), and *Shigella spp.* was positive in 2 (1%). Of the 50 fresh, raw, unpasteurized milk samples collected from various districts of Ankara province, gram-negative bacilli (*E. coli*, *Klebsiella*) was isolated from 22, Group-D *Streptococcus* was isolated from 7, *Diphtheroid* was isolated from 5, coagulase-negative *staphylococcus* was isolated from 22, and coagulase-positive *staphylococcus* was isolated from 4. In the present study, 2 *E. coli* isolates not fermenting sorbitol in SMAC, one from the stool and one from the milk samples, were isolated and they displayed negative reaction with *E. coli* O157 antiserum in the initial testing.

DISCUSSION

Verotoxigenic *E. coli* (VTEC) O157:H7 infections can appear in a wide clinical spectrum from asymptomatic course to the hemolytic uremic syndrome, which is a fatal clinical picture. Although the studies from Turkey reported the incidence of VTEC O157:H7 to be 0% - 11.6%, verotoxin could have not been determined or studied in these studies (Aydoğan, Sünbül, Leblebicioğlu, Eroğlu, & Esen, 2001; Yeniiz, Öncül, & Çavuşlu, 2009). The clinical course of VTEC infections is quite characteristic; watery stool accompanied by cramp-like abdominal pain occurs after 3-4 days of incubation period and the stool becomes bloody in the following days (Pennington, 2010). Absence of high body temperature and moderate abdominal pain must suggest *E. coli* O157:H7 (Karch, Tarr, & Bielaszewska, 2005). Slutsker et al. (1997) obtained 63% of *E. coli* O157:H7 isolated from bloody stools which was followed by *Shigella* (14.7%), *Campylobacter* (7.8%), and *Salmonella* (4.8%). The Centers for Disease Control and Prevention recommends investigation of *E. coli* O157:H7 routinely in stool cultures, at least in bloody stools (Gould et al., 2009). Erdoğan et al. (2011) detected *Entamoeba histolytica* in 6 (9%), *Salmonella spp.* in 2 (3%), and VTEC O157:H7 in 2 (3%) of 67 patients with macroscopic bloody stool. In the present study, EIEC was positive in 9 (4.5%), *Klebsiella spp.* was positive in 7 (3.5%), EPEC was positive 3 (1.5%), *Salmonella spp.* was positive in 2 (1%), and *Shigella spp.* was positive in 2 (1%) of bloody stool samples. Suspicious bacteria were detected only in one of 200 stool samples; however, as confirmation test showed negative result, the suspicious *E. coli* strain was considered not to be the serotype O157:H7.

The prevalence of isolating *E. coli* O157:H7 from milk has been studied in various regions of the world (Hill, Smythe, Lindsay, & Shepherd, 2012; Farahmandfar, Moori-Bakhtiari, Gooraninezhad, & Zarei, 2016). In the present study, gram-negative bacilli (*E. coli*, *Klebsiella*) was isolated from 22, Group-D *Streptococcus* was isolated from 7, *Diphtheroid* was isolated from 5, coagulase-negative *Staphylococcus* was isolated from 22, and coagulase-positive *Staphylococcus* was isolated from 4 of 50 fresh, unpasteurized raw milk specimens collected from various districts of Ankara province. Suspicious bacteria were isolated from only one of 50 milk specimens; however, this suspicious *E. coli* strain was considered not to be the serotype O157:H7 as confirmation test showed negative result.

E. coli O157:H7 is an uncommon bacterium (Rosensweig, & Gourley, 1991). The prevalence of isolating this bacterium shows differences among seasons and geographic regions. Prevalence of *E. coli* O157:H7 isolation has increased particularly in Canada, Northern US, and United Kingdom. Not only the contaminated foods but also inadequate implication of hygienic measures has led to increased *E. coli* O157:H7 isolation (Belongia et al., 1991). Shiga-like toxin produced by *E. coli* O157:H7 plays crucial role in pathogenesis. The present study aimed to determine the prevalence of isolating *E. coli* serotype O157:H7, which has recently gained importance among gastroenteritis agents, in the patients with bloody diarrhea and in raw milk samples. False-positive results can be obtained despite the use of ideal isolation techniques. In the present study, we investigated *E. coli* O157:H7 in 200 bloody stool samples and in 50 unpasteurized milk samples using ideal isolation techniques. We detected a total of two suspicious bacteria, one from 200 stool samples and one from 50 raw milk specimens. Confirmation tests yielded negative results; thus, we determined that the suspicious strains were not *E. coli* O157:H7.

E. coli O157:H7 has been isolated from some regions but not from other regions in some countries. The present study suggests the necessity of gathering and reviewing the outcomes of further studies that would be conducted in higher number of subjects from various regions of Turkey. The geographical condition of our country may be a factor for not isolating this agent. Taking hygienic measures during slaughtering, as well as cooking meat well-done and pasteurizing milk, prevents the growth of this bacterium. Slaughtering and controlling food of animal origin need to be performed carefully and precisely for the prevention of contamination with *E. coli* O157:H7, which can be found in many animals, food of animal origin and environment. We think that both the clinicians' and laboratory technicians' keeping this uncommon agent in mind especially in gastroenteritis cases with a history of bloody stool and eating suspicious food would be useful particularly for the prevention of fatal diseases caused by this agent.

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