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Microbiological Investigation of Stool in Patients with Acute Diarrhea

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Abstract: Acute gastroenteritis is an important health public issue especially in developing countries. The aim of this study was to determine the most common microbial agents responsible for acute diarrheas in our city. The study was performed prospectively between December 2005 and January 2006 in Manisa, Turkey. Stool samples were collected from patients with acute diarrhea who attended to different hospitals of the city (University Hospital, Government Hospitals, Pediatrics Hospital and Health Public Laboratory). The stool samples of 300 patients (50 children, 250 adults) were investigated. Standard cultivation methods were performed to determine *Salmonella* ve *Shigella* sp. *E. coli* 0157:H7 was tested by Immuno Card STAT! *E. coli* 0157 Plus. The strains that were identified as sorbitol negative *E. coli* were investigated by *E. coli* 0157: H7 antiserum. The isolated microorganisms were as follows: *Salmonella* sp. 7 (2.3%), *Shigella* sp. 5 (1.6 %), *G. intestinalis* 12 (4%), *Enterobius vermicularis* 5 (1.6%), *Rotavirus* 2 (0.6%) and sorbitol negative *E. coli* 18 (6 %). Only one Sorbitol negative *E. coli* colony showed agglutination with *E. coli* 0157:H7 antiserum. The most common pathogens were identified as *G. intestinalis* in children and *E. coli* in adults. Sorbitol negative *E. coli* were isolated in 18 of the stool samples, and only one of them showed agglutination with *E. coli* 0157:H7. Although rare, this strain may lead serious complications and it should be kept in mind in patients with acute diarrhea.

Key words: Diarrhea, *Escherichia coli* 0157, *Salmonella* sp., *Shigella* sp., *G. intestinalis*

INTRODUCTION

Acute gastroenteritis is an important health public issue especially in developing countries (Yildiz *et al*., 2005; Raji *et al*., 2003; Koneman *et al*., 1997). Viruses (50-70%), bacteria (15-20%) or parasites (10-15%) may be the causative pathogens. Rotavirus and adenovirus, Shigella, Salmonella and *E. coli* and Giardia and amebiasis are known as the most common ones. *E. coli* is the member of gastrointestinal microbial flora of humans and animals; however, it is known that many pathogenic *E. coli* serotypes may cause different conditions like hemorrhagic colitis, hemolytic uremic syndrome, trombotic thrombocytopenic purpura (Riley *et al*., 1983; Aksungur and Yaman, 1995; Coia, 1998; Akarsu *et al*., 2001). *E. coli* 0157:H7 first identified as a human enteric pathogen in 1982, has an important place among agents that cause acute infectious diarrheas (Riley *et al*., 1983). Although rare, diseases due to *E. coli* 0157 are serious and life-threatening (Aksungur and Yaman, 1995). The aim of this study was to determine the most common microbial agents responsible for acute diarrheas in our city.

MATERIALS AND METHODS

This prospective study was performed between December 2005 and January 2006. Stool samples were collected from patients with diarrhea who attended to different hospitals (University Hospital, Government Hospitals, Pediatrics Hospital and Health Public Laboratory) of Manisa, a city located in the western region of Turkey with 300 000 population. The patients who had soft defecation at least thrice a day were included to the study. The stool samples of 300 patients (50 children, 250 adults) were investigated. Direct microscopic examinations of the samples were performed and then they were cultivated in bloody agar, Eosin Methylene Blue (EMB) agar and Mac Conkey Agar with Sorbitol (MCAS) (Oxoid). Standard cultivation methods

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were performed to determine *Salmonella* ve *Shigella* sp. (Coia, 1998). Specific anti-sera were used for *Salmonella*(DIFCO Laboratories, Detroit, Michigan, USA), for *Shigella* (DENKA-SEIKEN Co, 3-4-2 Nihonbashi Kayabacho, Chuo-ku, Tokyo 103-0025 Japan) and for Rotavirus (CORIS, Rotastrip, Genbloux. Belgium).

E. coli 0157:H7 was tested by ImmunoCard STAT! *E. coli* 0157 Plus (Meridian Bioscience, Inc., Cincinnati, Ohio, 45244). Following the incubation for a night at 37°C, passages of sorbitol negative colonies were performed to bloody agar and Mac Conkey Agar with Sorbitol. After that, biochemical tests were performed. The strains that were identified as sorbitol negative *E. coli* were investigated by *E. coli* 0157:H7 antiserum (DENKA-SEIKEN Co, 3-4-2 Nihonbashi Kayabacho, Chuo-ku, Tokyo 103-0025 Japan) (Mackenzie *et al.*, 2000).

RESULTS

The samples were collected from 300 patients-50 (16.6%) from children and 250 (83.4%) from adults. The ages of the patients ranged from 1 to 80. Of adult patients, 120 (47.1%) were male and 130 (52.9%) were female. The mean age was 36.2±18.6 (15-80). Of children, 33 (66%) were male and 17 (34%) were female. Their mean age was 4.26±2.18 (1-9).

Microscopic examination revealed leukocytes in 81 (27%) and erythrocytes in 48 (16%) of the samples. The isolated microorganisms were as follows: *Salmonella* sp. 7 (2.3%), *Shigella* sp. 5 (1.6%), *G. intestinalis* 12 (4%), Rotavirus 2 (0.6%) and sorbitol negative *E. coli* 18 (6%). Only 1 (0.33%) sorbitol negative colony showed agglutination with *E. coli* 0157:H7 antiserum. This sample was obtained from a 4-year old patient who had soft bloody defecation. Two sorbitol negative colonies were obtained from children, the others belonged to adult patients (9 male, 7 female) (Table 1).

Table1: The pathogens isolated from patients with acute diarrhea

	Adult N=250	%	Children N=50	%	Total N=300	%
Salmonella sp.	5	2	2	4	7	2.3
Shigella sp.	4	1.6	1	2	5	1.6
Rotavirus	-		2	4	2	0.6
Sorbitol negative *E.coli*	16	6.4	1	2	17	5.6
E. coli 0157:H7	-		1	2	1	0.33
Giardia intestinalis	9	3.6	3	6	12	4

DISCUSSION

In this study, the most common pathogens were identified as *G. intestinalis* in children and *E. coli* in adults. Different agents have been reported as the most common pathogens from different countries. De Witt *et al.* (2001) from Netherlands reported rotavirus and Norwalk-like virus in small children, *Campylobacter* sp. and *G. lamblia* in older children and *Campylobacter* sp. in adults. In a study from Australia (Sinclair *et al.*, 2005) the most common pathogens were Norovirus virus (10.7%), pathogenic *E. coli* 0157:H7 (6.7%), *Campylobacter* sp. (3.0%) and *Giardia* sp. (2.5%). Rotavirus was reported to be the most common agent (26.6 and 40%, respectively) in children with acute diarrhea (Ali *et al.*, 2005; Rosenfeldt *et al.*, 2005). Ali *et al.* (2005) reported *Salmonella* in 13.6%, *Shigella* in 3.6% and *G. lamblia* in 1.2%. Chan *et al.* (2003) from China reported that *Vibrio parahaemolyticus* as the commonest bacterial pathogen in adults; ratios for *Salmonella* sp. and *Shigella* sp. were 34.6 and 6.2%, respectively. According to studies from our country, rates for *Salmonella* sp. and *Shigella* sp. have been reported as 1.1, 10.2 and 1%-10.8%, respectively (Kenan and Aksit, 2003; Chan *et al.*, 2003). *Salmonella* sp. was identified in 2.3% and *Shigella* sp. was identified in 1.6% of the samples in this study.

E. coli 0157:H7 was isolated in an outbreak hemorrhagic colitis in 1982; the most important virulence property of this serotype is to produce Shiga toxin 1 and Shiga toxin 2 (Bartly, 1990). Many *E. coli* 0157 colonies produce Shiga toxin 2. *E. coli* 0157 infection is important because: It can spread from human to human, may lead to serious complications and there is no specific treatment (Taylor and Blaster, 1991). *E.coli* 0157:H7 contamination may occur by uncooked veal, unpasteurized milk and milk products, dirty water or by direct contact with infected animals (Bartly, 1990; Taylor and Blaster, 1991; Cobeljic *et al.*, 2005; Brandt *et al.*, 1994). *E. coli* 0157 infections are widespread worldwide. All ages can be affected but the disease is more serious in children and elderly. It has been reported that up to 15% of Hemorrhagic Colitis (HC) cases, Hemolytic Uremic Syndrome (HUS) characterized by hemolytic anemia and renal failure may develop; however, the mechanism of HC and HUS due to *E. coli* 0157:H7 is not well known (Lewinson and Jawetz, 2004; Margeret and Yungbyuth, 1994). Sorbitol negative *E. coli* was isolated in 106 (4.1%) of 2552 patients in one study and defined as

E. coli 0157 (Harris *et al*., 1985). *E. coli* 0157:H7 was detected in 0.1% of 2889 patients with acute diarrhea in a study from Japan (Taguchi *et al*., 1989). Different studies from our country reported the sorbitol negative *E. coli* positivity between 0.6 and 14.14% (Sen *et al*., 2002; Aksunger *et al*., 1995); *E. coli* 0157:H7 was detected in 0.75%-4% of patients with diarrhea (Aksungur and Yaman, 1995; Sen *et al*., 2002; Guney *et al*., 2001) although in some studies *E. coli* 0157:H7 was not isolated (Tolun *et al*., 2001; Akca *et al*., 1996; Halepliler and Babur, 1993; Hascelik *et al*., 1991; Erensoy and Tokbas, 1992).

In this study, sorbitol negative *E. coli* were isolated in 18 of the stool samples, and only one of them showed agglutination with *E. coli* 0157:H7. Although rare, this strain may lead serious complications and it should be kept in mind in patients with acute diarrhea.

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