LETTER-TO-THE-EDITOR

No Isolation of Escherichia coli O157:H7 Strains from Faecal Specimens of Turkish Children with Acute Gastroenteritis*

Sir:

Several strains of Escherichia coli that produce relatively large amounts of cytotoxin active on Vero or HeLa cell, termed as verocytotoxin, are recognized as important aetiological agents of diarrhoea in children, particularly in developed countries. Most of these recognized E. coli strains belong to serotype O157:H7. This organism, first isolated in the USA in 1982, has been associated with both outbreaks and sporadic cases of human disease, ranging from uncomplicated diarrhoea to haemorrhagic colitis and haemolytic-uraemic syndrome in North America (1,2). The reported frequency of E. coli O157:H7 infections continues to increase, probably reflecting both a greater interest in this pathogen and a real increase in its incidence and geographic spread (3,4). To the best of our knowledge, no information on the prevalence of E. coli O157:H7 in Turkish population is available.

During 1994-2000, 649 faecal specimens collected from paediatric patients (234 females), aged six months to seven years, with bloody and watery diarrhoea were investigated. Most patients were originated from the same geographical area (Ankara region, central Anatolia) and had an urban way of life. The age distribution of the 649 patients was as follows: 6-12 months–147; 1-2 year(s)–186; 2-4 years–245; and 4-7 years–71.

Faecal specimens were suspended in phosphate-buffered saline with a proportion of 1/10 and then streaked out onto sorbitol (1%) MacConkey agar (SMAC) plates and incubated at 37 °C for overnight. From each sample, 5-10 colonies of non-sorbitol-fermenting colonial type were selected for identifying E. coli O157:H7 strain. Colonies were emulsified in a drop of saline on a glass slide and then mixed with one drop of E. coli O157:H7 antiserum (Difco, USA). Biochemical confirmation of non-sorbitol-fermenting colonies that were agglutinated with E. coli O157:H7 antisera was made, using an API ID32E test (BioMérieux, Marcy-L’Etoile, France).

For verotoxin production, reference strain (E. coli, 4288-84) and clinical isolates that were not fermenting sorbitol, but were agglutinated by E. coli O157:H7 antisera, were inoculated in 10 mL of trypticase soy broth and then incubated at 37 °C for overnight. The culture was then centrifuged (1,200 g, 5 minutes), and supernatant filtrates were sterilized with a 0.45-µm-pore-size filter (Sartorius AG, Germany). This filtrate was used directly for investigating verotoxicity in monolayers of Vero cells (African green monkey kidney cells). An un inoculated filtrate of trypticase soy broth was also used as control. After the addition of filtrate to Vero cell plates, they were covered and incubated for 24 hours at 37 °C in 5% CO₂ atmosphere. The monolayers were examined after two or three days for cytotoxic effects using an inverted microscope. Verotoxin production was determined by visualizing presence of cells that rounded up and became detached in the wells.

Although small, round-edged, transparent, gray colonies on SMAC plates suspected to be E. coli O157:H7 were detected in 183 (28%) specimens, 23 (3%) of them were agglutinated by E. coli O157:H7 antiserum. However, none of the isolates produced verotoxin. Additionally, biochemical characteristics of these 23 isolates correlated well with Escherichia hermannii, with a probability of 99.8%.

Most outbreaks in North America and in the UK due to verotoxin-producing E. coli O157:H7 (VTEC) have been associated with consumption of under-cooked hamburgers and other beef products. This organism is the fourth most commonly-isolated bacterial agent of diarrhoea in the USA (3). VTEC infections occur much less frequently in continental Europe than in North America and in the UK. Some European countries have reported several outbreaks due to VTEC, but other

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E. coli O157:H7 strains from faecal specimens of Turkish children

The prevalence of E. coli O157:H7 serotype in Turkey is not well-known, and VTEC was not isolated in Turkey until 1994, but two Austrian tourists returning from that country were found to have VTEC O157 infection (5-7). E. coli O157:H7 was not isolated in this study as was done in previously-reported studies from Turkey. Also, no study was conducted in cattle and other animals or their food products known to be a reservoir of E. coli O157:H7 in Turkey. Additionally, biochemical characteristics of our isolates correlated well with E. hermannii, with a probability of 99.8%. In the case of positive agglutination test with antiserum O157:H7 of isolates on SMAC agar with presumptive identification of VTEC, it should be kept in mind that E. hermannii does also not ferment sorbitol and may give false-positive agglutination test when using commercially-purchased antisera. Hence, E. hermannii can exhibit biochemical characteristics and serological cross-reactivity with E. coli O157:H7 (8,9). For this reason, we do not recommend routine screening of this pathogen by SMAC agar and antisera only. False presumptive positive results can be reduced by detection of verotoxin production.

This limited study indicates that Turkey may not be a harbouring country for this pathogen, because there are no data to show any isolate of E. coli O157:H7, possibly because of food tradition. From an epidemiological perspective, only major microbiological centres should continue a surveillance system to prevent emerging of this highly-dangerous pathogen.

REFERENCES


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