APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1992, p. 412–414
0099-2240/92/010412-03$02.00/0

Vol. 58, No. 1

Lack of Virulence Factors in *Escherichia coli* Strains of Enteropathogenic Serogroups Isolated from Water

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Received 14 August 1991/Accepted 25 October 1991

Thirty-eight *Escherichia coli* strains belonging to 14 human enteropathogenic serogroups were isolated from 33 of 208 water samples studied. No virulence factor or virulence-related gene sequences were found in any of the 38 strains analyzed. The results point out the importance of detecting specific virulence factors before incriminating water as a source of human diarrhea.

Some *Escherichia coli* serotypes belong to the indigenous flora of human intestines. However, other serotypes are consistently associated with virulence mechanisms that render the bacteria enteropathogenic for humans. On the basis of their pathogenic mechanisms, enteropathogenic *E. coli* strains are presently classified in four distinct groups: classical enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), and enterohemorrhagic *E. coli* (15).

Biological and immunological assays as well as specific genetic probes may be used in the identification of enteropathogenic *E. coli* strains isolated from fecal and environmental samples. Efforts have been made to detect enteropathogenic *E. coli* strains in water samples of different origins (5, 9, 13, 14, 22, 24).

We report here the isolation from water samples of *E. coli* strains belonging to enteropathogenic serogroups. However, the majority of these strains belonged to serotypes not commonly associated with human diarrhea in Brazil, and all of them lacked known virulence factors.

Two hundred eight water samples were collected by standard procedures (1). The origins of the samples are shown in Table 1. *E. coli* colonies were collected by two procedures. In one procedure, approximately 4 liters of a sample was filtered through a membrane filter (diameter, 293 mm). The membrane was vigorously shaken in an Erlenmeyer flask containing 100 ml of 1% peptone water. The Erlenmeyer flask was incubated at 25°C for 24 h, and the resulting bacterial growth was plated on MacConkey and salmonella-shigella agars. In the other procedure, one aliquot of each sample, removed before membrane filtration, was processed by the multiple-tube technique for fecal coliforms (1). After the confirmatory test for fecal coliforms in *E. coli* (EC) medium, bacterial growth was plated on eosin-methylene blue agar. Suspect *E. coli* colonies grown on the selective media were confirmed biochemically (8).

E. coli colonies were submitted to slide agglutination with polivalent and monovalent antisera (PROBAC do Brasil) against somatic antigens of EPEC (O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158) and EIEC (O28ac, O29, O112ac, O124, O136, O143, O144, O152, O164, and O167). Only antisera against the ETEC serogroups most frequently occurring in São Paulo were used (O6, O8, O25, O63, O78, and O139). Confirmation of O antigens and identification of H antigens (H1 to H50) were performed by tube agglutination with specific antisera (8).

HeLa cells were used to detect localized adherence (26) and invasiveness (17). Invasiveness was also detected with the Serény test (28). The production of heat-labile toxin (LT) and heat-stable toxin (ST) was analyzed by the indirect hemagglutination (21) and the suckling infant mouse (3) assays, respectively. The following *E. coli* strains were used as controls for the virulence assays: 0041-1/85 (serotype O111ab:H−), 0431-4 (serotype O64:H4), and TR 302/4 (O8:H?), producing localized, enteroaggregative, and diffuse adherences, respectively; strain 9/82 (serotype O28ac:H−) is invasive, and H10407 (serotype O78:H11) is toxigenic, producing LT and ST, but not invasive. *E. coli* K-12 C600 was used as a negative control in all assays.

DNA sequences related to invasiveness and to EPEC adherence factor (EAF), LT, and ST production were used as probes to detect homologous sequences in colony hybridization assays under high-stringency conditions (16). The LT probe was the 1.2-kb *Hin*cII fragment from plasmid pEWD299 (19). The ST probes were the 240-bp *Hpa*II fragment from pSLM004 (18) and the 157-bp *Hin*fI fragment from pRIT10036 (19). The EAF probe was the 1.0-kb *Bam*HI-*Sal*I fragment from pMAR2 (20), and the invasiveness probe was the 2.5-kb *Hin*dIII fragment from pSF55 (29). *E. coli* K-12 strains bearing the recombinant plasmids cited were used as positive controls. *E. coli* HB101 was used as a negative control.

A total of 38 *E. coli* strains belonging to enteropathogenic serogroups were isolated from 33 water samples. Thirty-seven colonies were isolated from nontreated water (recreational lakes, dams, mines, rivers, and wells), and 1 was isolated from treated water (swimming pool). These colonies belonged to 14 different serogroups; 8 were EPEC, 5 were ETEC, and 1 was EIEC. As a number of studies have demonstrated that enteropathogenic strains are limited to only certain serotypes within specific serogroups (10, 11, 15, 25, 30), we further tested the 38 *E. coli* strains to determine their serotypes. Twenty-six distinct serotypes were identified. Table 2 shows the numbers of strains belonging to each of the serotypes found. Except for two strains that belonged to EPEC serotype O127:H− and EIEC serotype O124:H−,

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TABLE 1. Types and origins of the 208 water samples studied

Type	Origin	No.
Nontreated	Recreational lakes	62
	Dams	19
	Mines	16
	Rivers	14
	Wells	9
	Artesian wells	6
Treated	Swimming pools	73
	Tap water	9

all the others belonged to serotypes usually not associated with human diarrhea.

None of the *E. coli* strains belonging to EPEC serogroups expressed localized adherence to HeLa cells. Similarly, none belonging to ETEC serogroups produced enterotoxins (LT or ST), and the single strain belonging to the EIEC serogroup did not produce keratoconjunctivitis in guinea pigs or invade HeLa cells.

None of the strains hybridized with the specific probes used. Therefore, the lack of virulence factor production was due to the absence of virulence-related genes. It is important to point out that enteropathogenic strains may lose virulence plasmids during the isolation procedures normally used (12).

Various studies have implicated water as a source of human contamination of enteropathogenic *E. coli*, but the strains were characterized as enteropathogenic only by their O antigens (4, 27, 31). In two of these studies, the authors observed that *E. coli* strains of the same serogroups were also isolated from diarrheic feces in an outbreak of gastroenteritis (27, 31) in addition to *E. coli* isolated from water.

TABLE 2. Serotypes of *E. coli* strains isolated from water

Suspect group	Serotype[a]	No.
EPEC	O26:H32	4
	O26:H42	1
	O55:NT	1
	O86:NT	3
	O125:NT	2
	O126:H6	2
	O126:H32	1
	O127:H−	1
	O127:H4	1
	O127:H7	1
	O127:H21	1
	O127:H38	1
	O128ab:H35	4
	O142:H38	1
	O142:H43	1
EIEC	O124:H−	1
ETEC	O6:H10	1
	O6:H49	1
	O8:H14	1
	O8:H40	1
	O25:H−	2
	O25:NT	1
	O128ac:H−	1
	O128ac:NT	2
	O139:H19	1
	O139:NT	1

[a] H−, nonmotile; NT, nontypeable.

With a more complete bacterial analysis, Ewing (7) was able to incriminate tap water containing *E. coli* of serotype O111ab:H− as the source of the diarrhea affecting a child and his father. A stronger association could have been determined with further characterization of the strains by an analysis of virulence factor production, but the methods were not available at that time.

Other studies detected enteropathogenic *E. coli* in water by testing the strains for enterotoxin production (6, 9, 14) or the presence of specific related DNA sequences directly in the samples (5). However, the authors did not determine the serotypes of the strains, so it is not known whether the reported strains would have been pathogenic for humans. Sack et al. (23) and Sato et al. (24) demonstrated ETEC in water, but the strains belonged to serotypes not associated with human diarrhea, and the significance of these findings is not known.

The present results suggest that the water samples studied were not a potential source of human infection by enteropathogenic *E. coli*. However, other studies have reported the occurrence of enteropathogenic *E. coli* in water samples. Rosenberg et al. (22) implicated the ingestion of water containing enterotoxin-producing *E. coli* of serotype O6:H16 as the source of an outbreak of diarrhea. Black et al. (2) reported ETEC of the same serotypes in feces and the residential water of diarrheic patients. The infection rate during the study period was greater in residences in which contaminated food or water was found. Jiwa et al. (13) isolated enterotoxin-producing *E. coli* of serogroup O68 from water. This serogroup was frequently isolated from infantile diarrheic feces in the same community, but the serotype was not determined.

The multiple-tube technique detected 34 *E. coli* strains, whereas the membrane filtration technique detected only 4 strains. None of these 38 strains were detected simultaneously by the two procedures.

The successful isolation of enteropathogens from water depends on the methods used; the methods should allow a good concentration of the microbiota and further selection of the desired microorganisms. In the present study, the low recovery of enteropathogenic *E. coli* after concentration in membrane filters suggests that other bacteria present in the samples inhibited the development of *E. coli* colonies, since no specific method was used for *E. coli* selection.

In summary, the incrimination of water as a source of enteropathogenic *E. coli* depends on the method used for bacterial isolation as well as the definitive characterization of the isolates, which should include the detection of specific virulence factors.

This research was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo, Conselho Nacional de Desenvolvimento Científico e Tecnológico, and Financiadora de Estudos e Projetos.

We thank Marisa Fernandes and Regina Giraldi for technical assistance.

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