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Evaluation of Antisera Used for Detecting Enterotoxigenic *Escherichia coli* in Sao Paulo

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The usefulness of antisera in detecting enterotoxigenic *Escherichia coli* (ETEC) strains in Sao Paulo was evaluated. Polyvalent antisera detected 49% of ETEC isolates and were more effective in identifying *E. coli* that produced heat-labile and heat-stable enterotoxins and in strains that produced only heat-stable enterotoxin. ETEC strains not detected by the antisera belonged to different serogroups not isolated in Sao Paulo before; 34% of these strains had undetermined O antigens, and most of them produced only heat-labile toxin. A variation of serogroups over time was especially observed among strains that produced heat-stable toxin. The importance of H-antigen determinations in the effectiveness of ETEC diagnosis by serological methods became evident, as non-ETEC strains were also detected by polyvalent antisera, but their serotypes were different from those of ETEC strains. Although antisera can be used to identify O:H types of ETEC strains with accuracy, serotyping cannot be recommended for routine diagnosis. However, such a procedure may be useful for studying outbreaks of ETEC diarrhea if the involved serotypes are already known.

Enterotoxigenic *Escherichia coli* (ETEC) plays an important role in endemic and epidemic diarrhea in infants and adults, especially in developing countries (13, 17, 21, 22, 24). These strains can produce heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST) or both. Several biological and immunological tests for detecting LT production have been described (5, 9, 10, 11, 15, 26, 28), and for ST the most frequently used test is the infant mouse test (3), although the enzyme-linked immunosorbent assay (ELISA) (29) and DNA probes (12, 19) can also be used. As the tests used to detect ST require animals or special techniques and apparatus, routine laboratories usually do not search for ST-producing *E. coli*. As the relationship between the enterotoxigenic phenotype and the serogroup or serotype of ETEC strains has been demonstrated by many authors (2, 4, 6, 18, 20, 23), Merson et al. (16) proposed the use of polyvalent antisera to simplify the detection of ETEC. Compared with enterotoxin testing, the antisera had a sensivity of 64% and a specificity of 96% and were considered to be useful in the identification of ETEC strains.

The aim of this work was to evaluate the usefulness of antisera in detecting ETEC strains isolated in Sao Paulo and to compare antisera to classical methods used to detect enterotoxin production.

MATERIALS AND METHODS

Bacterial strains. A total of 516 *E. coli* strains isolated from the feces of 322 children with diarrhea and 78 controls attending different outpatient clinics were studied from 1980 to 1982. Feces were plated on MacConkey agar (Difco Laboratories) within 3 h after collection. For both patients and controls, five *E. coli* colonies grown on MacConkey agar plates were studied. When colonies from the same patient had the same biochemical characteristics or the same enterotoxigenic phenotype, they were considered to be a single strain. All strains were studied simultaneously for enterotoxin production and by serological testing in polyvalent antisera.

Enterotoxin assays. All *E. coli* isolates were tested for LT and ST production by the Y1 adrenal cell culture assay (5) and the infant mouse assay (3), respectively. The strains were cultivated with aeration in yeast extract medium (8) for both enterotoxin assays.

Preparation of antisera. For antiserum production, 12 *E. coli* strains belonging to the serogroups most frequently found among ETEC stains were selected. Monovalent O antisera were prepared in rabbits by the Roschka method with heated and dehydrated antigen (7). Before use, monovalent antisera were titrated with the vaccine stains. Polyvalent antisera I (O25, O62, O78), II (O8, O15, O60), III (O6, O20, O63) and IV (O128ac, O139, O148) were prepared by mixing the monovalent antisera in proportions that gave good slide agglutination results with the appropriate vaccine strains. During this study, the efficacy of polyvalent antisera was regularly monitored.

Serogroup and serotype identification. Heated suspensions (100°C, 30 min) of *E. coli* colonies in EPM medium (30) were used in slide agglutination tests. The suspensions were tested in polyvalent antisera I to IV, and if agglutination occurred in one of them, the corresponding monovalent antisera were used. The O antigens of the strains were confirmed by tube agglutination (7). The H-antigen determination was done by tube agglutination of actively motile cultures (7); H1 to H49 antisera were kindly provided by the Centers for Disease Control, Atlanta, Ga.

The ETEC strains whose serogroups were not identified by the above-described procedures were serotyped by standard methods (7) at the Centers for Disease Control with O1 to O164 antisera.

RESULTS

Enterotoxin assays. The Y1 adrenal cell culture assay and the infant mouse assay detected ETEC strains in 34 of 400 children studied. One child harbored two ETEC strains. Of these strains, 9 (26%) produced only LT, 19 (54%) produced only ST, and 7 (20%) produced LT and ST (Table 1).

Detection of ETEC by polyvalent antisera. The polyvalent antisera detected 49% (17 of 35) of all ETEC strains. The frequency of detection of each enterotoxigenic phenotype is

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TABLE 1. Detection of ETEC strains by polyvalent antisera and enterotoxin assays

Toxin type	Total no. of ETEC strains detected by enterotoxin assays	No. (%) of ETEC strains detected by polyvalent antisera
LT	9	1 (11)
ST	19	11 (58)
LT and ST	7	5 (71)
Total	35	17 (49)

shown in Table 1. These strains were restricted to serogroups O6, O25, O63, O78, and O128ac and belonged to specific serotypes which are regularly related to toxin types (Table 2). The 18 ETEC strains not detected by the polyvalent antisera did not belong to the selected serogroups. These strains belonged to 13 different serotypes (Table 2) not isolated in Sao Paulo before, except for serotypes O112:H$^-$ and O114:H21.

In addition to ETEC strains, 63 non-ETEC strains were detected by the polyvalent antisera. The most frequent serotypes were O6:H31 (15 strains), O8:H4 (4 strains), O15:H18 (3 strains), O20:H34 (7 strains), and O128ab:H35 (4 strains). Different serotypes were found among the ETEC and non-ETEC strains belonging to the same O serogroup (Table 3).

DISCUSSION

In the present work it was verified that antisera prepared against the most frequent ETEC O serogroups detected 49% of ETEC isolates that had been identified by enterotoxin assays. The frequency of detection was different for each enterotoxigenic phenotype; 71% of the strains produced LT and ST, 58% produced only ST, and 11% produced only LT. Such results were expected, as strains that produce both LT and ST are generally restricted to a smaller number of serogroups (2, 4, 6, 18, 23), and in Sao Paulo strains that produce only ST belong to a smaller number of serotypes than those that produce only LT (6, 18, 22, 23).

In a recent study conducted in two Aboriginal communities in tropical northwestern Australia, Berry et al. (1) observed that the polyvalent antisera proposed by Merson et al. (16) would have detected only 3 of 58 ETEC strains isolated.

TABLE 2. Toxin types and serotypes of ETEC strains detected or not detected by polyvalent antisera

Toxin type (no. of strains)	Serotype[a] (no. of strains)	
	Detected by polyvalent antisera	Not detected by polyvalent antisera
LT (9)	O78:H10 (1)	O64:H$^-$ (1), O88:H25 (1), O112:H$^-$ (1), O114:H21 (1), ND:H2 (1), ND:H8 (2), ND:H39 (1)
ST (19)	O78:H12 (3), O128ac:H7 (2), O128ac:H12 (4), O128ac:H27 (2)	O27:H$^-$ (3), O27:H7 (2), O29:Hsp (2), ND:H16 (1)
LT and ST (7)	O6:H16 (2), O25:H42 (1), O63:H$^-$ (2)	O23:H$^-$ (1), ND:H10 (1)

[a] H$^-$, Nonmotile; ND, undetermined O1 to O164; Hsp, spontaneous agglutination.

TABLE 3. ETEC and non-ETEC O:H types belonging to the same O serogroup

Serogroup	O:H type[a]	
	ETEC	Non-ETEC
O6	O6:H16	O6:H$^-$, O6:H1, O6:H31
O25	O25:H42	O25:H$^-$, O25:H1
O63	O63:H$^-$	O63:H$^-$
O78	O78:H10, O78:H12	O78:H$^-$
O128	O128ac:H7, O128ac:H12, O128ac:H27	O128ab:H$^-$, O128ab:H2, O128ab:H35

[a] H$^-$, Nonmotile.

Stoll et al. (27), comparing the serogroups of 207 ETEC strains isolated in Bangladesh in 1980 with results obtained from similar surveys conducted in 1976 and 1978, showed that during this period of time, the distribution of serogroups changed significantly. They found that only 46% of their strains had O serogroups included in polyvalent antisera capable of detecting 64% of the ETEC strains in 1978. Similar results were obtained in this work, as all ETEC strains which were not identified by antisera were distributed among several serogroups not isolated in Sao Paulo before, except for serogroups O112 and O114. Moreover, 34% of these ETEC strains had undetermined O antigens, and most of them produced only LT. A high frequency of nottypable ETEC strains was also described by Georges et al. (12) in a study conducted in the Central African Republic. These observations probably indicate that such strains belong to serotypes not previously identified as ETEC (18, 20, 23).

Our results and data from others (20, 23, 24) suggest that some serogroups have a universal distribution, whereas others are more frequent in certain geographical areas; furthermore, a change in serogroups over time in the same place (27) has also been observed. Therefore, one must consider geographical and local diversities of serogroups when any group of antisera is used to identify ETEC strains.

The association of enterotoxigenicity with specific serotypes or bioserotypes has been well demonstrated by many authors (14, 18, 20, 23–25) and has been confirmed in the present study. Table 3 shows that the toxigenic serotypes were all antigenically different from the nontoxigenic ones, except for serotype O63:H$^-$. However, the toxigenic and nontoxigenic strains of this serotype were completely different in biochemical tests (data not shown).

Although serological and biochemical methods can be used to identify O:H types of ETEC strains with accuracy, bioserotyping cannot be recommeded for routine diagnosis. However, such a procedure may be useful for studying outbreaks of ETEC diarrhea if the bioserotype involved has already been determined.

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