

Virulence Properties and Characteristics of Shiga Toxin-Producing *Escherichia coli* in São Paulo, Brazil, from 1976 through 1999

T. M. I. Vaz,¹ K. Irino,¹ M. A. M. F. Kato,¹ Â. M. G. Dias,¹ T. A. T. Gomes,²
M. I. C. Medeiros,³ M. M. M. Rocha,⁴ and B. E. C. Guth^{2*}

Instituto Adolfo Lutz¹ and Universidade Federal de São Paulo—Escola Paulista de Medicina,² São Paulo,
Instituto Adolfo Lutz, Ribeirão Preto,³ and Instituto Adolfo Lutz, Campinas,⁴ Brazil

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Twenty-nine Shiga toxin-producing *Escherichia coli* (STEC) strains were identified in a collection of 2,607 isolates from patients with diarrhea in São Paulo, Brazil, from 1976 to 1999. The STEC strains belonged mainly to serotypes O111:HNM (HNM, nonmotile) (13 of 29 [44.8%]), O111:H8 (7 of 29 [24%]), and O26:H11 (4 of 29 [13.8%]); *stx*₁ *eae* (26 of 29 [89.6%]), in combination with either enterohemorrhagic *E. coli* *hlyA* (11 of 26 [42%]) or *astA* (24 of 26 [92.3%]), prevailed. The O111 STEC strains were distinguished by their inability to decarboxylate lysine. The predominance of STEC O111 and O26 since the late 1970s and the identification of STEC serotypes O55:H19, O93:H19, and O118:H16 in association with human infections in Brazil are described for the first time.

Shiga toxin-producing *Escherichia coli* (STEC) can cause a broad spectrum of human illness ranging from uncomplicated diarrhea to hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) (14). *E. coli* O157:H7 is by far the most important STEC serotype in the world and has been associated with numerous outbreaks and many sporadic cases of HC and HUS (20). However, infections with other non-O157 STEC serotypes have been increasingly reported in many countries (2, 3, 22). Currently more than 100 different non-O157 STEC serotypes have been isolated from human infections. Unlike with O157:H7, outbreaks caused by non-O157 STEC have rarely been reported, and these strains have been more frequently associated with sporadic cases of diarrhea, HC, and HUS. Moreover, in many countries, non-O157 strains are more commonly isolated from patients with diarrhea or HUS than are O157 STEC strains (3, 5, 23). The major virulence trait of these bacteria is the production of one or more Stx types (Stx1, Stx2, or Stx2 variants) (20). Additional factors, produced mainly by strains in straight association with human disease and collectively referred to as enterohemorrhagic *E. coli* (EHEC), include intimin and enterohemolysin (1, 20). Other putative virulence factors include the enteroaggregative *E. coli* heat-stable enterotoxin (EAST-1), encoded by *astA* (25), particularly in O157:H7 and O26:H11 strains and in some other STEC serotypes. In Brazil, the studies conducted so far that searched for STEC in human diarrheal disease have investigated a limited number of strains and have described the isolation of a few non-O157 STEC strains (7, 12, 16). The isolation of O157:H7 strains from patients with bloody diarrhea as well as one with HUS related to O26:H11 infection was only recently reported in Brazil (15, 19).

The purpose of this study was to search for STEC in a collection of 2,607 *E. coli* isolates, most of them belonging to

important serogroups increasingly associated with HUS and HC in other countries. The main virulence factors, biotypes, and susceptibility to antimicrobial agents of the STEC strains identified were characterized.

The *E. coli* strains studied belonged to the collection of Instituto Adolfo Lutz, a Central Public Health Laboratory in São Paulo, Brazil, and national center for *E. coli* serotyping, and were isolated between 1976 and 1999, mainly from children (0 to 5 years old) with diarrhea (2,549 strains); the remaining 58 *E. coli* strains were isolated from immunocompromised adult patients with diarrhea (mainly human immunodeficiency virus positive) over the same period. Each strain was derived from one patient. Except for diarrhea, no other information regarding the clinical aspects of the patients were available. None of the strains belonging to the enteropathogenic *E. coli* (EPEC) serogroups carried the EAF sequence (EPEC adherence factor) as assayed by hybridization assays with a specific DNA probe (13). The remaining strains outside the EPEC serogroups did not belong to the enteroinvasive *E. coli* (EIEC) serogroups, as determined by agglutination assays with specific antisera (10), and did not produce enterotoxins characteristic of the enterotoxigenic *E. coli* (ETEC) category (20).

The *stx*₁, *stx*₂, *eae*, EHEC *hlyA*, and *astA* sequences were sought by colony hybridization assays with specific DNA probes as described previously (13, 28). *E. coli* strains carrying *stx*₁ and/or *stx*₂ were tested for cytotoxicity activity by a Vero cell culture assay (11). The serotypes were determined by standard agglutination assays using O1 to O173 and H1 to H56 antisera (10), prepared at Instituto Adolfo Lutz. Production of enterohemolysin was determined by the method of Beutin et al. (1). The detection of fermentation patterns and the lysine decarboxylation test were performed by standard methods (6, 10). The susceptibility to antimicrobial agents was determined by the standard disk diffusion method (21) using the following antibiotics (Cecon, Centro de Controle e Produtos para Diagnóstico Ltda, São Paulo, Brazil): ampicillin, amikacin, cefepime, cefoxitin, tetracycline, gentamicin, ceftazidime,

* Corresponding author. Mailing address: Disciplina de Microbiologia, Universidade Federal de São Paulo, Rua Botucatu 862/3 andar, CEP 04023-062, São Paulo, SP, Brazil. Phone: 55-11-5576-4537. Fax: 55-11-5571-1095. E-mail: becguh@ecb.epm.br.

TABLE 1. Frequency of STEC among several *E. coli* serogroups isolated from patients with diarrheal disease in São Paulo, Brazil, from 1976 to 1999

| Serogroup ^a | No. of strains studied | No. (%) of STEC strains |
|------------------------|------------------------|-------------------------|
| O26 | 205 | 4 (1.95) |
| O55 | 209 | 1 (0.48) |
| O111 | 802 | 21 (2.61) |
| O113 | 16 | 0 |
| O119 | 305 | 0 |
| O157 | 15 | 1 (6.67) |
| ND | 1,055 | 2 (0.19) |
| Total | 2,607 | 29 (1.1%) |

^a ND, undetermined (not belonging to the EPEC, EIEC, and ETEC categories).

ceftriaxone, imipenem, trimethoprim-sulfamethoxazole, tobramycin, kanamycin, chloramphenicol, ciprofloxacin, and streptomycin.

STEC strains were identified in 29 (1.1%) of the 2,607 *E. coli* strains analyzed, and the frequency varied among the different serogroups studied (Table 1). Most (27 of 29 [93%]) of the STEC strains were isolated from children (45% from infants aged 0 to 12 months and 34% from children aged 1 to 5 years), but two of them were from adults. The STEC strains belonged mainly to serotypes O111:HNM (13 of 29 [44.8%]), O111:H8 (7 of 29 [24%]), and O26:H11 (4 of 29 [13.8%]), but serotypes O55:H19, O93:H19, O118:H16, and O157:H7 (one strain each) were also detected (Table 2). The great majority of the strains (93%) harbored *stx*₁, while the only O157:H7 strain carried *stx*₂, and the *stx*₁ *stx*₂ genotype was observed in the O93:H19 strain. Cytotoxin production was confirmed in all strains containing *stx*. Except for two strains, all the others carried *eae*, and the EHEC *hlyA* gene was identified in 13 (44.8%) of the 29 strains. Moreover, it could be observed that STEC strains were isolated in 1976 and in subsequent years, but no predominance in a particular year was identified (Table 2). Analysis of the biochemical behavior of the STEC strains showed that all O111 STEC strains fail to decarboxylate lysine whereas this reaction was positive in the other serotypes identified. Moreover, lack or delay in fermenting sucrose was observed only in serogroup O111. All strains except the O157 strain fermented sorbitol, and inability to ferment rhamnose and dulcitol was associated with O26 and O118 strains, which

were also the only ones that fermented sorbose. Resistance to at least one drug was identified in 51.7% of the STEC strains, and occurred among strains of serogroups O26 (2 strains), O111 (12 strains), and O118 (1 strain). However, multidrug resistance (to three to six drugs) was observed only in serotypes O111:HNM (four strains) and O111:H8 (one strain). The antibiotics for which resistance was most frequently observed were tetracycline (93%), streptomycin (73%), kanamycin (33%), chloramphenicol (20%), and trimethoprim-sulfamethoxazole (13%) (either alone or in combination).

In the present study we observed the predominance of O111 and O26 serotypes among STEC strains identified in São Paulo from 1976 through 1999 and described for the first time the association of other serotypes such as O55:H19, O93:H19, and O118:H16 with human infections in Brazil. The great majority of these non-O157 serotypes are among those most frequently isolated in many different countries (2, 9, 14, 23). In São Paulo, low rates of occurrence O111 STEC strains had previously been reported (16). Although outbreaks of HUS caused by O111 strains had been described (4, 5, 8), the O111 STEC strains identified so far in our settings were all associated with sporadic cases of diarrhea in young children. Moreover, the results of this retrospective study and the recent description of the isolation of a STEC O26:H11 strain associated with HUS (15) are in contrast to previous reports that described the nonoccurrence of STEC among O26 strains in Brazil (24, 26, 27).

In many developed countries, O157:H7 is the most prevalent STEC serotype associated with severe disease in humans (14, 22). However, estimation of the prevalence of O157-associated infections in Brazil is difficult since screening for O157 is performed by only a few microbiological laboratories. The only O157:H7 strain found in the present study, isolated in 1990 from a human immunodeficiency virus-positive patient, and the two other O157:H7 strains that we recently isolated in São Paulo are the only O157:H7 strains identified so far in association with human disease in Brazil (19).

In this study *stx*₁ *eae* prevailed, and an association between *eae* and EHEC *hlyA* was seen among strains of serotypes O26:H11, O111:H8, O111:NM, O118:H16, and O157:H7. Thus, considering the serotypes and virulence profiles, these STEC strains can be classified as EHEC, and it would not be surprising if some of them had been associated with more severe diseases. We can only speculate about this possibility, because

TABLE 2. Serotypes, virulence markers, and year of isolation of STEC strains isolated in São Paulo, Brazil

| Serotype (no. of strains) | <i>stx</i> gene type | Presence of (no. of strains): | | | Presence of EHEC Hly (no. of strains) | Yr of isolation (no. of strains) |
|------------------------------|-------------------------|-------------------------------|------------------|-------------|--|--|
| | | <i>eae</i> | EHEC <i>hlyA</i> | <i>astA</i> | | |
| O26:H11 (4) | 1 | + | + | + | + | 1981, 1985, 1987, 1997 |
| O55:H19 (1) | 1 | - | - | - | - | 1984 |
| O93:H19 (1) | 1 + 2 | - | + | - | + | 1984 |
| O111:NM (13) | 1 | + | + (3) | + (12) | + (3) | 1978 (3), 1980 (2), 1981, 1983, 1985, 1986, 1987, 1988 (2), 1993 |
| O111:H8 (7) | 1 | + | + (3) | + (6) | + (2) | 1976, 1977, 1979, 1985, 1988, 1993, 1998 |
| O111:H11 (1) | 1 | + | - | + | - | 1981 |
| O118:H16 ^a (1) | 1 | + | + | + | + | 1989 |
| O157:H7 ^a (1) | 2 | + | + | + | + | 1990 |

^a Isolated from adults.

of the retrospective character of the present study and because detailed clinical data for the patients are not available.

With regard to the biochemical behavior of the STEC strains, besides confirming that all STEC O111 strains were lysine decarboxylase negative, as previously reported by Guth et al. (16, 17), we observed that this reaction also distinguished O111 STEC strains from the other STEC serotypes identified. The inability to decarboxylate lysine and the lack of sucrose fermentation by the majority of the O111:HNM strains showed that these strains can be misidentified presumptively as EIEC or as *Shigella*. The inability to ferment rhamnose was recently reported as a characteristic of O26 STEC isolates (18). Nevertheless, among the strains presently identified, O118 as well as O26 was rhamnose negative. In conclusion, the results of this study showed that potential EHEC strains, particularly O111 and O26 strains, have been found in São Paulo since the late 1970s. The implementation of a systematic surveillance of bloody diarrhea and HUS in Brazil will contribute to estimations of the real association of O157:H7 and non-O157 strains with human infections.

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