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Prevalence of Enterotoxigenic *Escherichia coli* Strains Harboring the Longus Pilus Gene in Brazil

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The longus type IV pilus gene (*lngA*) was highly prevalent (32.8%) among Brazilian enterotoxigenic *Escherichia coli* strains producing both heat-labile and heat-stable enterotoxins and bearing the CFA/I, CS1CS3, or CS6 antigen. Furthermore, *lngA* was more often found in strains isolated from children with diarrhea than in strains isolated from children without diarrhea.

Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of acute childhood diarrhea in developing countries (15). The production of heat-labile enterotoxin (LT) and/or heat-stable enterotoxin (ST) is responsible for the loss of fluids in the intestine. In addition, another important characteristic of these strains is the ability to colonize the small intestine, mediated by appendages called colonization factors (CFs). A spectrum of different surface filaments has been described among ETEC strains on the basis of their biochemical and immunological properties (3). Longus is a type IV pilus produced by human ETEC strains and is closely related to the CFA/III antigen (4, 13). Type IV pili are observed in a variety of gram-negative bacterial pathogens including *Vibrio cholerae* and enteropathogenic *E. coli* (5, 7, 25). It has been proposed that the most prevalent CFs are the best components for oral ETEC-based vaccines (2, 29). However, the prevalence of CFs among clinical ETEC isolates differs considerably depending on the geographic region of the world where the isolates are recovered (1, 11, 17, 20, 23, 26).

ETEC strains are important pathogens in São Paulo, Brazil, and are responsible for 7 to 20% of cases of infantile diarrheal (8, 21). Different studies have shown that CFs occur in 43 to 29% of the strains isolated, but a considerable number of ETEC strains isolated from patients with diarrhea still do not produce any of the known CFs (11, 16, 21). No data concerning the frequency of occurrence of longus pilus-producing strains in São Paulo are available. In the present study, the occurrence of the longus pilus was sought in a collection of 140 ETEC strains isolated from patients with or without diarrheal disease during several studies conducted in São Paulo from 1985 to 1990 (8, 10, 11). The toxigenic phenotypes, serotypes, and CF profiles of these strains have been determined previously (11, 16). Sera with polyclonal antibodies against CFA/I, CFA/II (CS1CS2, CS1CS3, CS2), CS8, and CFA/IV (CS6) (11) and CS18 (kindly provided by Glória Viboud [28]) or monoclonal antibodies (MAbs) against CS3, CS4, CS5, CS7, CS12, CS14, and CS17 (kindly provided by Ann-Mari Svernnerholm [27]) were used in dot blot enzyme-linked immunosorbent assays for CF identification. ETEC strain E9034A, which carries a 90-kbp plasmid with the CS3 and longus pilus genes, and strain E9034P, a plasmidless derivative of E9034A that is unable to produce the longus pilus, were used as positive and negative controls, respectively.

Colony blot hybridization and PCR were used to detect the longus pilin gene (*lngA*). Two specific probes, a 1.0-kbp *Eco*RV fragment from plasmid pOG140 containing the entire structural subunit *lngA* gene (9) and a 0.6-kbp *Eco*RI fragment from plasmid pZG1, were labeled with [α-^{32}P]dCTP and used in colony hybridization experiments as described previously (14). Oligonucleotides JG1 (5′-CGGAATTCATGAGCCTGCTGG AAGTTATCA-3′) and JG2 (5′-CGGAATTCCGGCTACCT AAAGTAATTGAGT-3′) were used to amplify a 630-bp amplicon representing the entire *lngA* gene. Bacterial lysates were obtained from colonies on MacConkey agar that had been suspended in distilled H_2O and boiled for 10 min. The amplification conditions were applied as described previously (12).

The longus pilus-related sequence (*lngA*) was identified in 32.8% (46 of 140) of the ETEC strains studied, and it was mainly observed among LT type I (LT-I)- and ST type I (ST-I)-producing strains and ST-I-producing strains (Table 1). This frequency is higher than those described for strains from other geographic regions such as Bangladesh, Chile, and Argentina, where it varies from 8.5 to 28%, but it was similar to the ones identified for strains from Egypt (31%) and Mexico (36.5%) (6, 12, 18). *lngA* has mostly been associated in previous studies with ST-I-producing strains (40 to 64.4%), and this percentage is in agreement with the one found in the present study. However, in this study the highest prevalence of *lngA* was observed among LT-I- and ST-I-producing strains (75%).

Most of the longus pilus-positive ETEC strains (69.5%) also harbor other CFs (Table 2), including CFA/I (53%), CS6 (21.8%), and CS1CS3 (18.7%). In other countries, *lngA* was most highly associated with CFA/II-producing strains and even with strains lacking any of the known CFs (6). In São Paulo, *lngA* was more prevalent among strains expressing CFA/I. Such an association was also found in ETEC strains isolated in Argentina (18). An association of CFs is not a common property of ETEC strains except for an association between CS6

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TABLE 1. Occurrence of *lngA* in ETEC strains with different toxigenic phenotypes isolated in São Paulo, Brazil[a]

Toxin type produced	No. of ETEC strains studied	No. (%) of strains ETEC *lngA* positive
LT-I	66	5 (7.5)
LT-II	5	0
ST-I	47	26 (55.3)
ST-II	2	0
LT-I and ST-I	20	15 (75)
Total	140	46 (32.8)

[a] The toxigenic phenotypes were determined in previous studies (11, 16). Except for two ST-I-producing strains and two LT-I- and ST-I-producing strains isolated from children without diarrhea, all other strains were isolated from children with diarrhea. ETEC strains producing only LT-II or ST-II are infrequently found in studies conducted in São Paulo (11).

and CFA/III, CS12, and CS22, but this association occurs at a low frequency. However, it should be mentioned that a high degree of association between CS6 and the longus pilus (21.8%) was observed in the present study, suggesting heterogeneity among the strains expressing CS6. Taking into consideration the frequencies of occurrence of the widespread CFs, such as CFA/I, CFA/II, and CFA/IV, which have varied from 23 to 43% in previous epidemiological studies conducted in São Paulo (11, 16, 21) and from 22 to 73% in studies conducted in other geographic regions (1, 17, 20, 23), the frequencies of occurrence of the longus pilin genes found here or described by others confirm the importance of the longus pilus as a widely distributed antigen among ETEC strains. Moreover, almost all (91%) of the *lngA*-positive ETEC strains identified in the present study were isolated from children with diarrhea, suggesting an association of the presence of *lngA* with disease. Girón et al. (6) have also observed an association between the presence of *lngA* and disease.

Interesting observations were also made when the *lngA*-positive ETEC strains were further analyzed for toxigenic phenotypes, serotypes, and expression of CFs (Table 3). LT- and ST-producing *lngA* strains were associated with only CFA/I or CFA/II (CS1CS3, CS2CS3) production, and such strains were only of serotypes O6:H16 and O78:H12. In addition, all of the longus pilus- and CFA/II-positive strains produced LT and ST. This strong association could be explained by the fact that these factors could be encoded on the same plasmid or on compatible plasmids. *lngA* was distributed among several serotypes of ST-producing strains, but a close association with CFA/I or CS6 production was also detected. In other regions of the world, the relationship between *lngA*, enterotoxin production, and CFs has mainly been associated with LT and ST production plus CFA/II and ST production and CFA/I production (6, 12, 18). Despite the relation of longus pili and the production of other CFs that have been identified, *lngA* also occurred in a relevant percentage (30.5%) of ETEC strains that lack CFs, suggesting that the longus pilus is a rather common antigen among the ETEC strains isolated in our community.

TABLE 2. Frequency of occurrence of other CFs observed among *lngA*-positive ETEC strains identified in Brazil[a]

Toxin type produced	No. of *lngA*-positive strains	No. (%) of strains with:				
		CFA/I	CS1CS3	CS2CS3	CS6	Total
LT-I	5	1	0	0	1	2
ST-I	26	10	0	0	6	16
LT-I and ST-I	15	6	6	2	0	14
Total	46	17 (53)	6 (18.7)	2 (6.2)	7 (21.8)	32 (69.5)

[a] CS1, CS2, and CS3 are part of the CFA/II fimbrial family, while CS6 is part of the CFA/IV fimbrial family.

TABLE 3. Relationship between *lngA* and the toxin profiles, serotypes, and CFs of ETEC strains isolated in Brazil

Toxin type produced	Serotype[a] (no. of strains)	CF[b] (no. of strains)
LT-I	R:H32	−
	O159:H21	−
	O78:12 (2)	CS6 (1)
	O6:NM	CFA/I
ST-I	O12:NT	CFA/I
	O27:NM (2)	CS6 (2)
	O27:H7 (2)	CS6 (2)
	O28:H8	CFA/I
	O77:H7	CS6
	O78:H12 (2)	CFA/I (2)
	O153:H45 (9)	CFA/I (4)
	O163:H33	CFA/I
	O165:H9	CFA/I
	O169:NM	CS6
	NT:H25	−
	NT:H8	−
	NT (3)	−
LT-I and ST-I	O6:H16 (10)	CFA/I (1)
		CS1CS3 (6)
		CS2CS3 (2)
	O78:H12 (5)	CFA/I (5)

[a] R, rough; NM, nonmotile; NT, nontypeable.
[b] −, negative for CFs CFA/I, CFA/II (CS1CS2, CS2CS3, CS2), CFA/IV (CS4CS6, CS5CS6, CS6), CS7, CS8, CS12, CS14, CS17 and CS18 (11,16).

All of the data discussed here point to the notion that the longus pilus frequently occurs among ETEC strains in different geographic regions, and this should be taken into consideration when strategies for the prevention of ETEC infections such as the use of pilus-based vaccines are considered. In addition, an antibody response against the longus pilus was detected in sera and fecal extracts of patients with diarrhea caused by ETEC (19), suggesting that this fimbria may be expressed in vivo. Since diarrheal disease due to ETEC is an important cause of childhood morbidity and mortality, prophylactic intervention to prevent infections with these strains should be discussed. It has been shown that CFs are good ETEC immunogens that can elicit a protective antibody response (22, 24). Thus, for the purpose of vaccine formulation studies, searches for the distribution and prevalence of CFs in areas of endemicity as well as further surveys of the human immune response against ETEC infections are essential.

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