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Adhesin-Encoding Genes from Shiga Toxin-Producing *Escherichia coli* Are More Prevalent in Atypical than in Typical Enteropathogenic *E. coli*▽

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Four of six adhesin-encoding genes (*lpfA*, *paa*, *iha*, and *toxB*) from Shiga toxin-producing *Escherichia coli* strains were detected in typical and atypical enteropathogenic *E. coli* (EPEC) strains of various serotypes. Although the most prevalent gene was *lpfA* in both groups, *paa* was the only potential diarrhea-associated gene in atypical EPEC.

The enteropathogenic *Escherichia coli* (EPEC) pathotype is subdivided into typical EPEC (tEPEC), which carries the large virulence EPEC adherence factor (EAF) plasmid (pEAF), and atypical EPEC (aEPEC), which lacks this plasmid (14). The pEAF encodes the bundle-forming pilus (BFP), which mediates a localized adherence (LA) pattern on HeLa/HEp-2 cells (13).

Both EPEC subgroups produce attaching and effacing (A/E) lesions on enterocytes, a phenotype associated with the chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE). Intimin is an outer membrane protein encoded by the *eae* gene and is the fundamental LEE-encoded adhesin determining establishment of A/E lesions (reviewed in reference 13).

The LEE is also found in enterohemorrhagic *E. coli* (EHEC), a subgroup of the Shiga toxin-producing *E. coli* (STEC) pathotype (19). Various additional adhesins have been described in STEC, including Saa (STEC autoagglutinating adhesin) in LEE-negative STEC strains (18); Paa (porcine A/E-associated adhesin), involved in the initial adherence of porcine EPEC strains (4); Lpf (long polar fimbriae), an adhesin found in EHEC and other pathogenic *E. coli* (26, 27); ToxB (EHEC pO157 plasmid-encoded protein), which is involved in adherence of EHEC O157:H7 (24); Iha (IrgA homologue adhesin), an adhesin similar to *Vibrio cholerae* IrgA (23); and EspP (extracellular serine protease), which contributes to bovine intestinal colonization (9).

aEPEC strains are a highly heterogeneous group, with many strains presenting an assorted repertoire of virulence genes from various *E. coli* pathotypes (8, 11, 20, 25, 30). Interestingly, aEPEC strains carrying certain virulence genes or specific combinations are significantly associated with diarrheal disease (2, 11, 20, 21, 30). There is evidence indicating that some aEPEC strains are in fact tEPEC or EHEC strains, which spontaneously lost either pEAF or the Shiga toxin-encoding genes (*stx* genes) (28). Furthermore, some comparative studies of the aEPEC genome have shown a closer relationship with EHEC than with tEPEC (16, 28), and others have shown that some clonal aEPEC groups are not related to any of these pathotypes (25).

In previous studies, we have searched for a number of *E. coli* adhesin-encoding genes in our EPEC collection, including the STEC *efa1/lifA* gene, but none of them were prevalent (11, 29,

TABLE 1. Adherence patterns and FAS test results from 100 aEPEC strains isolated from 73 patients and 27 controls

Phenotypic characteristic[a]	No. (%) of strains from:		
	Patients	Controls	Total
LAL	60 (82.2)	19 (70.4)	79 (79.0)
FAS$^+$	55 (75.3)[b]	14 (51.8)[b]	69 (69.0)
FAS$^-$	5 (6.8)	5 (18.5)	10 (10.0)
LAL/AA	3 (4.1)	0	3 (3.0)
FAS$^+$	3 (4.1)	0	3 (3.0)
FAS$^-$	0	0	0
AA	3 (4.1)	1 (3.7)	4 (4.0)
FAS$^+$	2 (2.7)	1 (3.7)	3 (3.0)
FAS$^-$	1 (1.3)	0	1 (1.0)
DA	2 (2.7)	4 (14.8)	6 (6.0)
FAS$^+$	2 (2.7)	2 (7.4)	4 (4.0)
FAS$^-$	0	2 (7.4)	2 (2.0)
NC	3 (4.1)	2 (7.4)	5 (5.0)
FAS$^+$	2 (2.7)	2 (7.4)	4 (4.0)
FAS$^-$	1 (1.3)	0	1 (1.0)
NA	1 (1.3)	1 (3.7)	2 (2.0)
D	1 (1.3)	0	1 (1.0)

[a] FAS, fluorescent actin staining; LAL, localized adherence-like; AA, aggregative adherence; DA, diffuse adherence; NC, noncharacteristic adherence; NA, nonadherent; D, cell detachment.

[b] Difference was statistically significant ($P = 0.0302$).

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TABLE 2. Prevalence of STEC adhesin-encoding genes in tEPEC and aEPEC strains from patients and controls

Adherence gene	No. (%) of strains:					
	aEPEC[a]			tEPEC[b]		
	Patients	Controls	Total	Patients	Controls	Total
lpfA	43 (58.9)	16 (59.3)	59 (59.0)	20 (50.0)	4 (66.7)	24 (52.2)
paa	35 (48.0)[c]	7 (25.9)[c]	42 (42.0)[d]	6 (15.0)	0	6 (13.0)[d]
iha	22 (30.1)	7 (25.9)	29 (29.0)[e]	3 (7.5)	1 (16.7)	4 (8.7)[e]
toxB	4 (5.5)	2 (7.4)	6 (6.0)	1 (2.5)	1 (16.7)	2 (4.35)
espP	3 (4.1)	0	3 (3.0)	0	0	0
saa	0	0	0	0	0	0
None	7 (9.6)	8 (29.6)	15 (15.0)	17 (42.5)	1 (16.6)	18 (39.1)

[a] The numbers of aEPEC strains studied were 73 from patients and 27 from controls.
[b] The numbers of tEPEC strains studied were 40 from patients and 6 from controls.
[c] $P = 0.067$.
[d] $P = 0.0005$.
[e] $P = 0.0058$.

30). Therefore, we aimed at investigating the prevalence of six other STEC adhesin-encoding genes and their potential association with diarrhea in aEPEC strains and compared them with those of tEPEC strains.

We examined a total of 146 *E. coli* strains, which were isolated from 113 diarrheic and 33 nondiarrheic children and adults in Brazil. The strains were characterized as EPEC based on the presence of the *eae* and absence of the *stx* genes (14).

TABLE 3. Distribution of STEC adhesin-encoding genes among aEPEC strains of distinct serotypes

Serotype	No. of strains	STEC adhesin-encoding gene(s)	*lpfA1* type	*lpfA2* type	Serotype	No. of strains	STEC adhesin-encoding gene(s)	*lpfA1* type	*lpfA2* type
O2ab:H45	1	*paa*	1		O177:H−	1			
O11:H2	1		2	1	NT:H−	3	*iha*		1
O11:H16	1					1			
O16:H−	1	*iha*				1	*toxB*, *iha*		
O19:H−	1	*iha*	1			1[c]	*espP*		
O26:H−	1		2	1		1		2	1
	3	*paa*	2	1		1	*paa*	1	
	1	*iha*				1	*iha*	1	
	1[a]	*iha*, *paa*, *espP*	2	1	NT:H2	1	*iha*		
O34:H−	1	*paa*	2	1	NT:H7	1[b]	*toxB*		1
	1	*iha*	2	1	NT:H8	1			
O39:H−	1[b]	*toxB*, *paa*				1[b]			1
O49:H−	1	*iha*		1		1[b]	*paa*	2	1
O49:H10	1[b]	*toxB*, *paa*				1		2	1
O51:H−	1	*iha*, *paa*	2	1		1			1
O55:H7	6	*paa*	3	2	NT:H9	1	*iha*		
	1	*paa*	3		NT:H11	1			
O63:H6	2	*paa*				1	*iha*		1
O85:H−	1	*iha*		1	NT:H19	1		2	1
O93:H−	1	*iha*, *paa*	2	1	NT:H25	1		2	1
O98:H8	1	*iha*, *paa*		1	NT:H29,31	1			
O101:H33	1				NT:H33	1[b]			
	1	*iha*			NT:H34	1		1	
O109:H9	1	*iha*		1		1	*iha*		
O111:H9	1					1	*paa*		
	1	*iha*		1	NT:H38	1			1
O119:H2	7[b]	*paa*	2		NT:H40	1			
O123:H19	1		2	1	NT:H40,43	1			1
O124:H40	1		1			1			
O125:H6	2				NT:H46	1	*paa*		1
O128:H2	4	*paa*			NT:NT	1[b]	*paa*, *toxB*		
O132:H8	2[b]	*paa*	2	1		1[b]	*paa*	1	1
O145:H−	1	*paa*			R:H−	1		1	1
O145:H34	1	*iha*, *paa*				1[c]	*iha*, *paa*, *espP*	2	1
O154:H9	1	*iha*		1	R:H11	1			
O157:H−	2	*iha*			R:H28	1		1	
O157:H16	2				R:H33	1	*iha*		
O160:H19	1		2	1	R:H40	1[b]			
O162:H−	1	*toxB*, *iha*							

[a] Strain carrying *ehxA* and *katP* genes.
[b] *bfpA*-positive strains lacking BFP production.
[c] Strain carrying *ehxA* gene.

Further classification as tEPEC or aEPEC was achieved by confirming BFP production by immunoblotting and by checking the *bfpA*-positive strains for their ability to produce LA on HeLa cells (after 3 h of incubation) (28). Strains lacking *bfpA* or carrying *bfpA* but lacking BFP production and showing an adherence pattern distinct from LA were classified as aEPEC (1, 12, 28). The aEPEC adherence patterns were defined after longer incubation periods (6 h) on HeLa cells (Table 1). The ability of all adherent strains to promote actin accumulation was evaluated by the fluorescent actin staining (FAS) test on HeLa cells (15). While all tEPEC strains were FAS positive (FAS^+) (data not shown), this property was detected in only 83% of the aEPEC strains isolated from patients and control subjects (Table 1). Interestingly, none of the aEPEC adherence patterns were associated with diarrhea, but FAS^+ strains producing the LA-like (LAL) pattern were statistically associated with diarrhea.

The presence of five STEC adhesin-encoding genes (*espP*, *toxB*, *saa*, *iha*, and *paa*) was examined by colony hybridization under stringent conditions, using as probes fragments of these genes obtained by PCR (3, 4, 6, 17, 22) and labeled with [^{32}P]dCTP. The presence of different *lpfA* alleles was investigated by PCR using primers and conditions previously described (26). Data were analyzed by two-tailed Fisher's exact test, and P values of ≤ 0.05 were considered statistically significant.

The *lpfA* genes were the most prevalent, followed by *paa*, *iha*, and *toxB* in both EPEC groups (Table 2). The *paa* and *iha* genes were significantly more frequent in aEPEC than in tEPEC strains, while *espP* was only found in three aEPEC strains, and *saa* was not detected (Table 2).

None of the adhesin-encoding genes were associated with diarrhea in both EPEC groups, but *paa* was more frequently found in aEPEC isolates from patients compared with isolates from controls, with this difference reflecting a trend to be statistically significant (Table 2). The association of *paa* with diarrhea has been previously observed in aEPEC isolates from Norwegian and Brazilian children (2, 21), but those studies did not examine tEPEC isolates.

Different combinations of the genes studied were found among diverse aEPEC and tEPEC serotypes (Tables 3 and 4). The distribution of *lpfA* in tEPEC agreed with our previous study (26); however, this is the first description of isolates *lpfA1-1* allele positive and *lpfA2* negative in serotype O142:H34 (Table 4). From the 100 aEPEC strains analyzed, 41 lack both *lpfA* alleles. Of the remaining 59 *lpfA*-positive strains, 20 contained the combination of *lpfA1-2* and *lpfA2-1* alleles. With the exception of one strain, all O55:H7 strains were *lpfA1-3* and *lpfA2-2*, thus confirming previous findings with EHEC O157:H7 and LEE-negative STEC strains (10, 26), that demonstrated that the presence of these two alleles is a reliable way to detect O157:H7 strains and the closely related O55:H− or O55:H7 serotypes. Although different combinations of these alleles were detected in aEPEC serotypes, we found that seven O119:H2 isolates carried the *lpfA1-2* allele and were negative for *lpfA2* (Table 3). Seven other isolates from different serotypes were also positive for the *lpfA1-1* allele and negative for *lpfA2*, and these alleles were found in rare tEPEC and aEPEC serotypes. Finally, a large number of EPEC strains were negative for both *lpfA1* and *lpfA2*, and no correlation with diarrhea was observed (Table 2).

TABLE 4. Distribution of STEC adhesin-encoding genes among tEPEC[a] strains of distinct serotypes

Serotype	No. of strains	STEC adhesin-encoding gene	*lpfA1* type	*lpfA2* type
O55:H−	1	*paa*	3	2
	1		1	
O55:H6	4		1	
	1			
O86:H34	1			
	1		1	
O88:H25	5		2	1
	1	*iha*	2	1
O111:H−	6			
O111:H2	6			
O119:H6	3	*paa*		
	3			
O127:H6	1	*toxB*	1	
O127:H40	1			
	1	*toxB*		
O142:H6	2	*iha*	1	
	2[b]	*paa*	1	
O142:H34	2		1	
	1	*iha*	1	
O145:H45	1		1,2	1
	2		1	

[a] *E. coli* strains carrying *eae* and *bfpA* and lacking *stx* genes, with all strains producing BFP.
[b] Only these two strains lacked the EAF probe sequence.

We also found that all strains of the traditional aEPEC serotypes O55:H7, O119:H2, and O128:H2 carried *paa* and all O119:H2 strains carried only *lpfA1-2* and lacked *lpfA2*. In contrast, the O26:H− strains showed different allele combinations which may reflect distinct H types (serotypes); however, with one exception, they possessed the *lpfA1-2* and *lpfA2-1* alleles. In addition, tEPEC strains of serotypes O111:H− and O111:H2, which were the most prevalent isolates in São Paulo, Brazil, in the past (28), carried none of the genes investigated.

The three strains that harbored *espP* (O26:H−, NT:H−, and R:H−) carried this gene on large plasmids, which also contained *ehxA*, although only one possess *katP* (Table 3). These findings suggest that these strains could comprise EHEC strains that have lost the *stx* genes (EHEC-LST) (5, 7). In six strains (two tEPEC and four aEPEC strains) that carried both *bfpA* and *toxB*, Southern blot analysis indicated that *toxB* is either located in pEAF or in another plasmid of similar size (data not shown).

In conclusion, we showed that a subgroup of aEPEC strains, producing the LAL pattern and FAS positivity on HeLa cells, are statistically associated with diarrhea. In addition, the prevalence of the STEC adhesin-encoding genes studied is higher in aEPEC than in tEPEC, possibly reflecting an apparently closer relationship of some aEPEC strains to the STEC pathotype. Our case control analysis showed a trend of the *paa* gene to be associated with aEPEC diarrhea. However, more studies are necessary to confirm that these genes are expressed during human infections and to understand how they contribute to host colonization.

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