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NOTES

Escherichia coli Strains of Serotype O51:H40 Comprise Typical and Atypical Enteropathogenic *E. coli* Strains and Are Potentially Diarrheagenic[▽]

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***Escherichia coli* strains of serotype O51:H40 were studied with regard to the presence of several virulence properties and their genetic diversity and enteropathogenicity in rabbit ileal loops. This serotype encompasses potential enteropathogenic strains mostly classified as being atypical enteropathogenic *E. coli* (EPEC) strains, which are genetically closer to enterohemorrhagic *E. coli* than to typical EPEC strains.**

Enterohemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *E. coli* (EPEC) comprise two of the six diarrheagenic *E. coli* pathotypes (16). While EHEC secretes Shiga toxin(s), EPEC adheres to HeLa/HEp-2 cells, forming compact clusters that distinguish the localized pattern of adherence (LA) (16). The LA is mediated by the bundle-forming pilus encoded by the EPEC adherence factor plasmid (pEAF) (16).

EHEC and EPEC share the ability to promote attaching-effacing (AE) lesions in intestinal cells (11) characterized by intimate bacterial adherence, localized microvillus effacement, and accumulation of polymerized actin and other cytoskeleton elements, resulting in pedestal-like structures underneath adherent bacteria (11). The AE lesion-associated genes reside in the locus of enterocyte effacement (LEE) (20), a pathogenicity island encoding a type III secretion system, regulators, chaperones, and effectors that interfere with diverse cell signaling processes (revised in reference 11). The LEE also encodes the outer membrane adhesive protein intimin (15) and its translocated receptor, Tir (18).

EPEC is currently subgrouped into typical EPEC (tEPEC) and atypical EPEC (aEPEC), where pEAF is present only in the former group (17, 34). Most tEPEC and aEPEC strains belong to defined (traditional) EPEC serogroups (34). Nevertheless, we previously identified and characterized several *E. coli* strains of non-EPEC serogroups isolated in three cities in Brazil carrying the intimin-encoding gene (*eae*) and lacking Shiga toxin-encoding genes (12). That study emphasized the large diversity of such strains but allowed no identification of the virulence potential of individual serotypes. Moreover, as both diarrheic and nondiarrheic patients may carry aEPEC strains (34, 36), it is important to evaluate the virulence potential of selected strains to identify those strains that are truly enteropathogenic.

In this study, we examined all 10 strains of serotype O51:H40, the most frequently found serotype in our previous study (12), and an additional uncharacterized O51:H40 strain (7) for novel *E. coli* virulence properties, their enteropathogenic potentials in vivo, genetic diversity, and genetic relatedness to tEPEC and EHEC. These results were compiled with those of our previous study (12) to attain an overview of the virulence potential of serotype O51:H40.

Table 1 presents the origins and the clinical and microbiological data of the patients carrying the 11 O51:H40 strains studied (7, 12, 13). Their adherence patterns in HeLa and differentiated intestinal Caco-2 cells were determined previously (12, 36), and in this study, strains were further tested in differentiated T84 cells as described previously (1). In HeLa cells, strain 0151-1 expressed LA after 3 h of infection, whereas seven strains presented an LA-like (LAL) pattern (27), and

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TABLE 1. Clinical, phenotypic, and genotypic characteristics of the O51:H40 strains studied

Strain	Age of patient	Other clinical information	Other pathogen(s)	Adherence pattern[g]	FAS[g]	TP[g,h]	AE lesions in rabbits[i]	Virulence gene(s)[j]	Antibiotic resistance(s)[k]	ERIC type
1757/01[b]	27 yr	None[e]	*Campylobacter* sp.	LAL	+	+	Yes	*eae shf*	None	IA1
21075[c]	8 mo	None	Rotavirus, DAEC	AA	+	+	Yes	*eae shf*	Amp Amc Sut Tet Azi	IA1
21323[c]	1 yr	None[f]	None	AA	+	+	Yes	*eae shf*	Amp Amc Sut Tet	IA2
1711-4[a]	4 yr 5 mo	Vomit, fever	DAEC	LAL	+	+	NT	*eae shf*	None	IB
1931-2[a]	2 yr 4 mo	Vomit, fever	Rotavirus	LAL	+	+	NT	*eae*	None	IIA1
3062-1[a]	2 yr 5 mo	Bronchitis[d]	None	LAL	−	−	No	*eae*	None	IIA1
3102-1[a]	1 yr 7 mo	Measles[d]	None	LAL	−	−	No	*eae*	Amp Amc Sut Tet	IIA1
2022/01[b]	1 yr	None	None	LAL	+	+	Yes	*eae*	None	IIA1
21242[c]	1 yr	Blood in feces	Rotavirus, DAEC	AA	−	+	Yes	*eae*	None	IIA1
4361-2[a]	1 yr 10 mo	Vomit	None	LAL	+	+	NT	*eae*	Amp Amc Sut Crx	IIA2
0151-1[a]	2 mo	Vomit, mucous in feces	None	LAL	+	+	NT	*eae* EAF *bfpA perA toxB*	None	IIB

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[d] Nondiarrheic patient.
[e] Patient with AIDS.
[f] Patient with persistent diarrhea.
[g] Tests performed in T84 cells for 6 h.
[h] TP, tyrosine phosphorylation of Tir.
[i] NT, not tested.
[j] Strains were reported previously to lack homology with gene probes for *E. coli* virulence factors STh, STp, LTI, INV, Stx1, Stx2, CDT, CNF, E-Hly, diffuse adherence, Afa, Sfa, Pap, enteroaggregative *E. coli* plasmid, EAST-1, aggregative adherence fimbriae I, II, and III, Hly, *aggR*, *irp-2*, *pic*, *aap*, and *pet* (12, 13, 36). All strains were positive for intimin subtype θ.
[k] As tested by the disc diffusion assay for 22 antibiotics. Amp, ampicillin; Amc, amikacin; Sut, sulfazotrim; Crx, cefuroxime; Tet, tetracycline; Azi, azithromycin.

three strains showed a noncharacteristic pattern in prolonged (6-h) assays (12, 36). In Caco-2 cells, all strains, except strain 21242, presented LAL patterns, whereas in T84 cells, eight strains (including 0151-1) presented LAL patterns, and three (strains 21323, 21242, and 21075) showed an aggregative adherence (AA) pattern (8) (Table 1). The potential to cause AE lesions was examined indirectly in the same cell lineages using a fluorescent actin staining (FAS) assay that detects actin accumulation (19). The divergent results among the three cell lineages could be due to different environmental factors (e.g., medium composition or the presence of specific receptors) or the induction of the expression of different bacterial adhesins. T84 was the most sensitive lineage, detecting eight (72.7%) FAS-positive (FAS^+) strains (Table 1), whereas only three (27.3%) and seven (63.6%) strains were FAS^+ in HeLa and Caco-2 cells, respectively (not shown). In T84 cells, all FAS^+ strains and one FAS-negative (FAS^-) strain promoted the tyrosine phosphorylation of Tir (28) (Table 1), thus behaving as tEPEC strain E2348/69, in contrast to EHEC strain EDL933 (9).

To verify the enteropathogenic potential in vivo, whole cultures of seven O51:H40 strains (three FAS^- and four FAS^+) were inoculated in rabbit ileal loops and examined by transmission electron microscopy (33). One FAS^- and all FAS^+ strains tested promoted AE lesions in this model (Table 1), confirming that at least five O51:H40 strains are potentially enteropathogenic. Interestingly, the two FAS^- strains (3062-1 and 3102-1) that were unable to promote AE lesions were isolated from nondiarrheic children. It is likely that mutations within the LEE (or their regulatory elements) rendered these strains nonpathogenic.

The O51:H40 strains were previously tested by colony blot or PCR for 29 diarrheagenic *E. coli* and extraintestinal pathogenic *E. coli* virulence genes (12, 13, 36). In this study, they were further tested for novel virulence genes (*efa1*, *saa*, *paa*, lpf_{O113}, *iha*, *toxB*, *ldaG*, and *agg3C*) by PCR (3, 5, 10, 21, 23, 29, 31, 32). Besides *eae*, four strains carried *shf*, and strain 0151-1 carried EPEC adherence factor, *bfpA*, and *perA* (which comprise pEAF sequences) as well as *toxB* (encoding an EHEC adhesin). Although the role of Shf in pathogenesis is unknown, the *shf* sequence has been detected in *Shigella flexneri*, EHEC O157:H7, and enteroaggregative *E. coli* strains (8). Its closest homolog is IcaB, a protein implicated in the intercellular adhesion of *Staphylococcus epidermidis* (14).

The plasmid profiles and antibiotic resistance patterns of the O51:H40 strains were analyzed by alkaline extraction (6) and disc diffusion agar (4), respectively. Although no common profile was found, a ~45-MDa plasmid band was observed in the three strains (not shown) that were resistant to ampicillin, amikacin, sulfazotrim, and tetracycline (Table 1), suggesting that it comprises a common resistance plasmid. Moreover, strain 0151-1 presented a 59-MDa band that hybridized with two pEAF sequences (EAF and *bfpA*) (2) as well as *toxB* (not shown). Since strain 0151-1 carried pEAF and expressed LA in HeLa cells, it was classified as being a tEPEC strain, whereas the remaining strains lacking pEAF and the *stx* genes and showing a LAL pattern were classified as being aEPEC strains.

LEE insertion sites vary according to the clonal origins of the *E. coli* strains (37). In tEPEC and EHEC strains, it is generally located adjacent to *selC* or *pheU* (26, 30, 37). However, as in the O51:H40 strains, these sites were intact, whereas

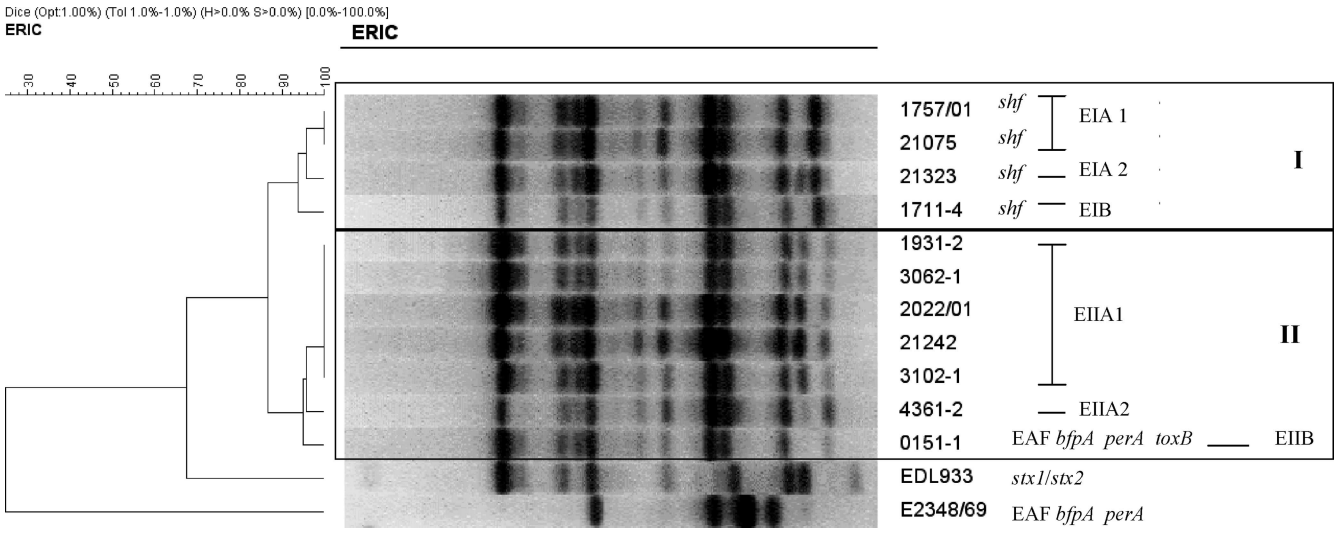

FIG. 1. Dendrogram of ERIC-PCR patterns of *E. coli* strains of serotype O51:H40 carrying *eae*. The analysis of similarity of the normalized images was accomplished using the unweighted-pair group method with arithmetic mean with the Dice optimized coefficient. Control strains were EPEC prototype strain E2348/69 and EHEC prototype strain EDL 933 (serotype O157:H7). Two different ERIC patterns (EI and EII) were found among the O51:H40 strains showing a similarity index of 87%.

when *pheV* was disrupted, the LEE is apparently located in *pheV* in these strains (12).

In animal models and in human intestinal biopsies, tEPEC preferentially colonizes the small intestines, whereas EHEC is restricted to the large intestine (24). Moreover, tropism to different colonization sites apparently depends on the intimin subtype (38). All O51:H40 strains carried the less common intimin subtype theta (Int-θ), as identified by PCR (12). Although the preferential adherence site of Int-θ has yet to be identified, it could comprise the small intestine since most O51:H40 strains tested promoted AE lesions in the rabbit ileum.

The genetic diversity of the O51:H40 strains and their relatedness to tEPEC and EHEC were investigated. Ribotyping (25) performed with BglI showed a single banding pattern, as expected by the fact that the strains belonged to a single serotype (22). However, enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) (35) generated six distinct electrophoretic types (E patterns) distributed in two main clonal groups, EI and EII, with a similarity of 87%, as calculated by the Dice unweighted-pair group method with arithmetic mean (Table 1 and Fig. 1). The genome differences found could reflect the horizontal acquisition of virulence factors (e.g., *shf*, *toxB*, and pEAF) or deletions. Interestingly, EI comprised four strictly correlated strains (all FAS and *shf* positive) despite their distinct geographical origins. Notwithstanding some heterogeneous characteristics within EII, five strains were identical by this technique. Note that ERIC-PCR showed a much closer relationship between the O51:H40 strains and EHEC strain EDL933 (68% similarity) than to tEPEC strain E2348/69 (<30% similarity). Although these findings corroborate a previous study showing that aEPEC strains are more related to EHEC than to tEPEC strains (35), they should be confirmed with a larger group of strains of both pathotypes.

In conclusion, serotype O51:H40 encompasses potential enteropathogenic strains mostly classified as being aEPEC strains, which are genetically more related to EHEC than to tEPEC strains.

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