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Distinct Pathotypes of O113 *Escherichia coli* Strains Isolated from Humans and Animals in Brazil[▽]

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Two distinct diarrheagenic *Escherichia coli* pathotypes, enteroaggregative *E. coli* (EAEC) and Shiga toxin-producing *E. coli*, were observed in association with O113 strains isolated from human and nonhuman sources in Brazil, respectively. The O113 strains from human diarrhea belonged to a diversity of serotypes, and nine (53%) of them harbored virulence traits of typical EAEC.

Diarrheagenic *Escherichia coli* (DEC) comprises six major pathotypes: enteropathogenic *E. coli*, enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli*, and Shiga toxin-producing *E. coli* (STEC) strains (12). Some of these groups have been well known and established as agents of disease for many years, while STEC and EAEC have emerged more recently as significant enteropathogens worldwide.

STEC strains are responsible for the occurrence of several diseases in humans, including the potentially lethal hemolytic-uremic syndrome. The production of Shiga toxin (Stx) is a key step in their virulence mechanism, but other factors, such as a hemolysin called EHEC hemolysin (Ehx) and the intimin protein, present in strains that harbor the *eae* gene, are also important (19). Although O157:H7 is the prominent STEC serotype, in the last decade many non-O157 STEC strains were also reported in diarrhea-associated illnesses and complications in several countries (19).

In fact, STEC serotype O113:H21 comprises one of these serotypes and is responsible for the occurrence of sporadic and small outbreaks of intestinal and extraintestinal diseases in different geographic areas (10, 17). Furthermore, the absence of *eae* in O113 STEC strains associated with hemolytic-uremic syndrome cases has led to investigations of other adhesins that may probably play a role in the pathogenesis of the diseases they can cause. Among them are some putative adherence proteins, such as the long polar fimbrial Lpf_{O113} and the non-fimbrial adhesin Saa (STEC autoagglutinating adhesin) (18). Other adherence factors, such as Iha (IrgA homologue adhesin) and ToxB, found mostly in *eae*-positive strains, may also occur in a minority of STEC O113 isolates (23).

On the other hand, EAEC is defined by a characteristic aggregative adhesion (AA) pattern on epithelial cells (12), and its association with acute and chronic diarrhea has increased steadily in recent years (9). This group shows a marked heterogeneity, and not all strains are believed to be pathogenic to humans. The term "typical EAEC" has recently been proposed to designate a subset of isolates that harbor the AggR transcriptional activator, which seems to be related to virulence (16, 20).

In Brazil, O113:H21 is one of the most frequent STEC serotypes isolated from cattle (3, 7, 11), but nonetheless, cases of human infections involving this STEC serotype have not been registered to date. However, O113 *E. coli* strains have already been recovered from patients with diarrhea in our settings (25). As no detailed information focusing only on serogroup O113 is available in the literature, the present study aimed to characterize and compare *E. coli* O113 strains isolated in Brazil from different sources and regions in relation to serotypes and several phenotypic and genotypic virulence markers, in an attempt to settle differences in their virulence potential.

A total of 38 O113 *E. coli* strains isolated during different surveys conducted in Brazil, from cases of human diarrhea ($n = 17$) (25; K. Irino, unpublished data), from cattle ($n = 19$) (3, 7, 11, 14), from buffalo ($n = 1$) (M. G. Oliveira et al., unpublished data), and from a meat sample ($n = 1$) (4), were studied. Only nonhuman O113 isolates were previously identified as STEC.

The O113 serogroup was confirmed in all strains, and the flagellar (H) antigens were determined by standard agglutination assays using H1 to H56 antisera as previously described (11). Nonmotile (HNM) strains were investigated for the flagellar gene (*fliC*) by restriction fragment length polymorphism-PCR (15). The presence of cytotoxic activity on Vero and HeLa cells, the production of enterohemolysin, and adhesion to HEp-2 cells were determined as previously described (8). Colony hybridization assays with specific DNA probes related to ETEC, EIEC, and EAEC pathotypes were performed under stringent conditions (26). The primers and conditions employed in the PCR assays for the identification of gene sequences related to stx_1, stx_2, *eae*, *ehxA*, *iha*, *saa*, lpf_{O113}, and *toxB* and for the subtyping of stx_1 and stx_2 were the same as those reported previously (1). PCR assays for the identification of EAEC genes and *aggR* were also performed (2, 16).

The O113 antigen was present in all isolates, and a diversity of H antigens could be identified (Table 1). H21 was the only antigen found in the STEC group and occurred in three (18%) of the isolates from human sources (non-STEC). Ten non-STEC strains were HNM, and the characterization of their *fliC* genes revealed two distinct restriction profiles that differed

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from those found in the H4, H7, H10, H12, and H21 strains (data not shown). The stx_2 and stx_{2c} genes, alone or combined, were found in 13 (62%) of the STEC isolates and occurred in association with stx_1 in the remaining strains. Adherence to HEp-2 cells was observed in 32 (84%) of the 38 strains studied, and the AA pattern was found in 29 (76%) of them (Table 1). The occurrence of a high percentage of AA among the studied strains, especially in those of the non-STEC group, suggested that these strains could belong to the EAEC pathotype. To test this hypothesis, hybridization assays were performed using DNA probe pCVD432 related to EAEC, but ETEC- and EIEC-specific probes were also assayed. None of the O113 strains hybridized with heat-labile enterotoxin I, heat-stable enterotoxin I, and invasion probes. However, 9 of the 38 (24%) isolates gave positive results with the EAEC probe, and all of them were non-STEC strains. These results were also confirmed by PCR, and except for one strain belonging to serotype O113:H12, all the others belonged to serotype O113:HNM (Table 1). The distributions of *eae*, *ehxA*, and *saa* and putative adhesin genes in the O113 isolates are shown in Table 1. Gene *ehxA* was identified only among STEC isolates (14 of 21, 66.6%). In addition, lpf_{O113} and *iha* were carried by all STEC isolates, and in the non-STEC group, these markers could be identified in six (35%) and nine (53%) of the isolates, respectively. All O113 isolates that were identified as EAEC carried *aggR*. The EAEC strains were isolated from nine diarrheic patients, children and adults, and in seven of them, *E. coli* O113 was the only enteropathogen isolated from stool culture. Moreover, five of the patients harboring EAEC presented AIDS (data not shown).

In the present study, O113 *E. coli* strains isolated from human and nonhuman sources in Brazil were shown to belong to distinct serotypes that were related to the EAEC and STEC categories, respectively. STEC serogroup O113, represented mainly by serotype O113:H21, is a recognized cause of human infections (2, 17). However, infections with EAEC serogroup O113 appear to be less common, and very few reports indicate the O113 serogroup in association with this DEC pathotype (22, 24).

It was interesting to observe that H21 was the unique antigen found among the STEC strains, which were all of animal origin. Only three human strains identified as non-STEC presented this antigen, whereas most of the EAEC strains were HNM. In many epidemiological surveys, H21 is commonly associated with STEC serogroup O113 (2), and to our knowledge, no other DEC pathotype has been described in association with the O113:H21 serotype. However, the loss of *stx* genes seems to be a common event (13), and one can suggest that the human O113:H21 strains analyzed may have lost these genes.

Studies have indicated that the stx_2 and/or stx_2 stx_{2c} genotype has been associated with more-severe human diseases (6). Although all the STEC serotype O113:H21 strains presently studied were of animal origin, the stx_2 and/or stx_{2c} genotype prevailed among the isolates. This result emphasizes the potential risk represented by cattle as natural reservoirs of highly virulent STEC serotypes in Brazil.

The distribution of the most common putative adhesin genes described in the STEC category showed that *saa* was found only among STEC isolates that presented the *ehxA* marker as

TABLE 1. Serotypes and phenotypic and genotypic virulence markers of *Escherichia coli* O113 strains isolated from human and nonhuman sources in Brazil[a]

Source	Serotype	Cytotoxic activity	Ehx	Adherence pattern(s)[b]	stx_1	stx_2	stx_{2c}		*ehxA*	*saa*	lpf_{O113}	*iha*	EAEC gene	*aggR*
							stx_{2vh-a}	stx_{2vh-b}						
Cattle	O113:H21 (19)	+	+ (13)	AA^3 (3), AA^6 (11), DA^3 (1), DA^6 (2), NA (2)	+ (7)	+	+ (5)	+ (6)	+ (13)	+ (13)	+	+	−	−
Buffalo	O113:H21 (1)	+	−	AA^3	+	+	+	−	−	−	+	−	−	−
Food	O113:H21 (1)	+	+	NA	−	+	−	−	+	+	+	+	−	−
Human diarrhea[c]	O113:HNM (10)	−	−	AA^3 (8), AA^6 (1), NA (1)	−	−	−	−	−	−	+ (1)	+ (6)	+ (8)	+ (8)
	O113:H4 (1)	−	−	AA^3	−	−	−	−	−	−	−	−	−	−
	O113:H7 (1)	−	−	AA^6	−	−	−	−	−	+	+	−	−	−
	O113:H10 (1)	−	−	AA^6	−	−	−	−	−	−	−	−	−	−
	O113:H12 (1)	−	−	AA^3	−	−	−	−	−	−	−	−	+	+
	O113:H21 (3)	−	−	AA^3 (1), NA (2)	−	−	−	−	−	+	+ (1)	−	−	−

[a] Numbers in parentheses indicate the number of strains. All strains tested were negative for *eae*- and *toxB*-related sequences.
[b] AA^3, aggregative adherence in 3-h assay; AA^6, aggregative adherence in 6-h assay; DA^3, diffuse adherence in 3-h assay; DA^6, diffuse adherence in 6-h assay; NA, nonadherent in 3- and 6-h assays.
[c] In 12 of the 17 cases of human diarrhea, *E. coli* serogroup O113 was the only enteropathogen isolated from stool culture. Nine of the 17 human strains were recovered from patients suffering with AIDS.

expected, since both genes are located in the STEC serogroup O113 megaplasmid (18). In addition, lpf_{O113} and *iha* occurred in all the STEC strains analyzed, confirming previous observations (23). Curiously, lpf_{O113} was also identified among non-STEC strains but did not occur in the EAEC group, while, in contrast, *iha* was observed at a high percentage (67%) only among the EAEC isolates.

The presence of AggR is being considered an indicator of EAEC strains that can cause disease and thus is being addressed as typical EAEC (9, 20). In accordance with this proposal, all the EAEC strains identified in this study could be classified as typical EAEC. Although the role of EAEC strains as agents of diarrheal disease in Brazil has already been described (21), this is the first report highlighting the occurrence of O113 EAEC strains in our settings and also from patients with AIDS. According to some studies, EAEC may be clinically important in AIDS, and its association with persistent diarrhea in human immunodeficiency virus-infected persons has been reported (5). Preliminary analysis of the genetic relatedness of the O113:HNM EAEC strains by pulsed-field gel electrophoresis revealed a diversity of profiles. Although most of the strains were grouped in the same cluster, a low degree of similarity was observed (less than 70%), except for two strains isolated from two different patients at an interval of 1 year that presented indistinguishable pulsed-field gel electrophoresis patterns (data not shown).

In conclusion, the O113 *E. coli* strains isolated so far from animals and humans in Brazil are related to two distinct pathotypes, STEC and EAEC, respectively. Nevertheless, the risk represented by cattle as reservoirs of O113 STEC to humans in Brazil should not be disregarded.

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