

Letters to the Editor

Enteroaggregative *Escherichia coli* Strains among Classical Enteropathogenic *Escherichia coli* O Serogroups

Enteroaggregative *Escherichia coli* (EAEC) is an emerging cause of diarrhea in both developing and developed countries (6) that is defined by the characteristic pattern of aggregative adherence (AA) to cultured epithelial cells (7). Studies from various laboratories have demonstrated that some enteropathogenic *E. coli* (EPEC) serogroups are comprised of serotypes mostly consisting of EAEC strains (2, 5, 9, 10). These serotypes present interesting characteristics: i.e., they have been isolated from cases of acute infantile diarrhea before AA was recognized as a distinct adherence pattern and in most cases express AA only in the 6-h adherence assay (5, 9, 10).

We examined our laboratory collection of *E. coli* strains that present the AA phenotype to HEP-2 cells and belong to the EPEC O serogroups (L. R. Trabulsi, unpublished data) for the presence of EAEC-associated virulence markers (3). Thirty-four AA-producing strains of the following serotypes were selected: O86:H2, O111:H4, O111:H10, O111:H12, O111:H21, O125:H6, O125:H16, O125:H21, O126:H27, O128:H12, and O128:H35. The strains were first analyzed for the presence of intimin (*eae*) and bundle-forming pilus structural subunit (*bfpA*) genes of EPEC (6). PCR for *eae* was performed as described earlier (1). The primers employed for *bfpA* detection (forward, 5'-GGTCTGTCTTTGATTGAATC-3'; and reverse, 5'-TTTACATGCAGTTGCCGCTT-3') were based on its published sequence (GenBank accession no. NC_002142) in order to amplify a 485-bp fragment. The presence of plasmid (*aggA*, *aafA*, *aggR*, *astA*, *pet*, *aspU*, and *shf*) and chromosomal (*irp2* and *pic*) EAEC-associated virulence markers (3) and the EAEC probe sequence (8) were also investigated by PCR, as previously described (3, 4, 8).

All strains evaluated were negative for the presence of EPEC virulence genes, except the two O125:H6 strains, which harbored the *eae* gene. Interestingly, these latter strains lacked all EAEC markers evaluated (Table 1) and presented the AA pattern to HEP-2 cells in the 6-h adherence assay. All other serotypes studied presented at least one EAEC marker (Table 1). The most prevalent markers found were *irp2*, *astA*, *shf*, and *pic*, which have also been described as prevalent among EAEC strains (3). The aggregative adherence fimbrial I pilin (*aggA*) gene (6) was the only EAEC marker not detected. This is in accordance with the low prevalence of *aggA* found in another study (4).

EAEC is very heterogeneous regarding the presence of putative virulence markers, and although some of these markers are frequent in the category, a sensitive gene probe for EAEC identification is not yet available (6). Accordingly, adherence to epithelial cells remains the “gold standard” diagnostic test for the category (7). Our results demonstrated that, with the exception of O125:H6, which has been classified as atypical EPEC (10), the serotypes studied here should be considered to be EAEC, since they presented the AA pattern and the majority of the EAEC-associated virulence genes. Studies in our laboratories are in progress in order to characterize the adhesin mediating the AA phenotype of the O125:H6 serotype and to ascertain whether these strains are able to produce the characteristic attaching-and-effacing lesion of EPEC (6). Our findings have clinical and epidemiological relevance, since EPEC identification by O serogrouping is misleading, as EPEC serogroups include different pathogens. Therefore, O sero-

TABLE 1. Detection of EAEC- and EPEC-associated virulence markers among *E. coli* strains belonging to EPEC serogroups and presenting the AA phenotype

Serotype	No. of strains (%)	No. of positive strains (%)											
		<i>eae</i>	<i>bfpA</i>	EAEC ^a	<i>aggA</i>	<i>aafA</i>	<i>aggR</i>	<i>aspU</i>	<i>shf</i>	<i>pet</i>	<i>astA</i>	<i>pic</i>	<i>irp-2</i>
O86:H2	4			4			4	3	3			3	3
O111:H4	1										1		
O111:H10	2			1	1		1	1				2	2
O111:H12	8				1						4	8	7
O111:H21	4			4			4	4			1	4	3
O125:H6	2	2											
O125:H16	1			1			1	1					1
O125:H21	1			1			1	1			1	1	1
O126:H27	2			2	1		1	1	1	1	1	1	2
O128:H12	1			1									
O128:H35	8								7		7		5
Total	34 (100)	2 (5.9)	0	14 (41.2)	0	2 (5.9)	12 (35.3)	11 (32.3)	19 (55.9)	1 (2.9)	22 (64.7)	17 (50)	24 (70.6)

^a EAEC probe sequence.

grouping should be complemented by flagellar (H) typing or, alternatively, by testing adhesion to epithelial cells.

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