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Evaluation of a Multiplex PCR for Identification of Enteroaggregative *Escherichia coli*[▽]

Enteroaggregative *Escherichia coli* (EAEC) has emerged as an important pathogen associated with endemic and epidemic diarrheal diseases in both industrialized and developing countries (6). EAEC strains are defined by their characteristic "stacked brick" aggregative adherence (AA) pattern to cultured epithelial cells (9), and this is the basis of the assay considered the gold standard for EAEC identification. However, this technique requires specialized facilities and can therefore be performed only in reference laboratories. As alternative to this technique, a variety of phenotypic and molecular assays have been proposed (1, 2, 4, 7, 15, 17). Recently, a multiplex PCR assay for EAEC detection has been developed (4). This assay detects simultaneously three EAEC plasmid-borne genes: *aggR*, which encodes a central regulator involved in the expression of several EAEC virulence genes (6, 10); *aap*, which encodes the antiaggregation protein dispersin (16); and *aatA*, which is part of a gene cluster that codes for a specific ATP-binding cassette transporter system (11). The latter corresponds to the EAEC probe fragment (CVD432) previously described (2).

In order to determine the specificity and sensitivity of multiplex PCR in detecting EAEC, a total of 379 fecal *E. coli* isolates were examined by this assay and the results subsequently correlated with the adherence pattern to HEp-2 cells. These strains were isolated from children under 5 years of age, with symptoms of gastrointestinal disorders, admitted to the Hospital Jesus, Rio de Janeiro, Brazil, between August 2003 and September 2004. PCR conditions and the primers used were the same as those described by Cerna et al. (4). All *E. coli* isolates were subjected to the adherence assay with cultured epithelial cells, employing a 3-h incubation of HEp-2 cells with bacterial cultures (5). Strains displaying noncharacteristic adherence (NC) or nonadherence (NA) were tested by employing an additional 3-h incubation of cells with bacteria (6-h assay).

At least one of the three loci sought was detected in 199 (52.5%) strains, while 180 (47.5%) strains were negative for them. The most prevalent was *aap*, detected in 153 (40.4%) strains, followed by *aggR*, detected in 109 (28.8%), and *aatA*, detected in 101 (26.6%). Concerning the adherence patterns observed, 158 (41.7%) strains showed the AA pattern and were therefore classified as EAEC (Table 1). Only four (1.1%) strains showed localized adherence (LA), which is a characteristic of enteropathogenic *E. coli* (14), while 156 (41.2%) showed an NC pattern and 61 (16.1%) were NA. Considering the AA pattern as the gold standard for EAEC identification, the detected loci were more frequent among EAEC strains, and the sensitivity and the specificity of the multiplex PCR were 93.5% and 81%, respectively. In addition, the positive predictive value was 75.2%, and the negative predictive value was 95.3%. None of the strains displaying the LA pattern amplified any of the primers tested. The three genes were also detected in strains displaying the NC pattern, and *aap* was detected in 10 NA strains. These NC and NA strains should be further analyzed in regard to the presence of additional plasmid and chromosome-associated EAEC virulence factors, since there is a possibility that they are EAEC strains that have lost the capacity to express the AA phenotype. The simultaneous detection of the three genes was most prevalent among the strains characterized as EAEC. Single or different combinations of two genes were also found preferentially in EAEC strains but also among the NC strains (Table 2). The detection of any of the three genes by multiplex PCR defines a strain as EAEC (4).

TABLE 1. Detection of EAEC plasmid-borne virulence factors by multiplex PCR in fecal *E. coli* strains in regard to their HEp-2 cell adherence patterns

Pattern of adherence[a]	No. of strains	No. (%) of each EAEC virulence gene detected by multiplex PCR		
		aatA	*aggR*	*aap*
AA	158	98 (62)	94 (59.5)	108 (68.4)
LA	4	0	0	0
NC	156	3 (1.9)	15 (9.6)	35 (22.4)
NA	61	0	0	10 (16.4)
Total	379	101 (26.6)	109 (28.8)	153 (40.4)

[a] Results of the 3- or 6-h adherence assay.

These three plasmid-borne genes detected by the multiplex PCR have been shown to be specific and appropriate markers for EAEC detection (3, 4, 7). In this study, multiplex PCR was shown to be sensitive and specific in detecting EAEC among fecal *E. coli* strains. Other previous published studies have also demonstrated that this technique is sensitive (3, 4). However, only strains previously characterized as EAEC, as defined by HEp-2 adherence, were evaluated in these studies. Additionally, two other multiplex PCRs employing different gene targets have been described for EAEC detection (8, 12), demonstrating that this technique is a useful tool in the detection of EAEC. However, both of the latter studies included the detection of *astA* (enteroaggregative heat-stable enterotoxin gene), which has been demonstrated to be present in other diarrheagenic *E. coli* pathotypes and nonvirulent *E. coli* (13). This may raise the possibility of the detection of *E. coli* strains that are not EAEC.

Since the technique evaluated in this study detects genes located on the high-molecular-weight virulence plasmid (pAA) of EAEC (4, 6), the inclusion of a chromosome-associated EAEC gene in the multiplex reaction, as performed by Jenkins

TABLE 2. Combinations of EAEC plasmid-borne virulence factors detected by multiplex PCR in fecal *E. coli* strains

Gene profile detected by multiplex PCR	No. of strains with gene profile with adherence pattern to HEp-2 cells (*n*)			
	AA (158)	LA (4)	NC (156)	NA (61)
aatA	21			
aggR	16		7	
aap	27		24	10
aatA aggR	2			
aatA aap	5		3	
aap aggR	6		8	
aatA aggR aap	70			
None	11	4	114	51

et al. (8), may improve its sensitivity/specificity, including the detection of EAEC strains that do not harbor pAA.

In conclusion, our results demonstrated that the multiplex PCR technique is a suitable alternative in detecting EAEC among *E. coli* strains isolated from feces, since PCR-based diagnostic tests are commonly employed in clinical laboratory practice.

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