SHORT ARTICLE

Distribution of *aggA* and *aafA* gene sequences among *Escherichia coli* isolates with genotypic or phenotypic characteristics, or both, of enteroaggregative *E. coli*

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Two types of fimbriae, designated aggregative adherence fimbria I and II (AAF/I and AAF/II) have been described in enteroaggregative *Escherichia coli* (EAEC) strains. These fimbriae mediate the aggregative pattern of adherence (AA) to epithelial cells. The genes encoding the structural subunit of each fimbria have been designated *aggA* and *aafA*, respectively. The prevalence of these genes was investigated in 155 faecal EAEC isolates that displayed AA to HeLa cells and were isolated from children in São Paulo, Brazil. Hybridisation assays with *aggA* and *aafA* sequences showed that 9.7% of these isolates carried *aggA*, 3.9% *aafA*, and none hybridised with both sequences. Of the 78 isolates displaying AA that reacted with the previously described EAEC probe (CVD432), 19.2% and 7.7% hybridised with the *aggA* and *aafA* probes, respectively. None of the 77 isolates displaying AA but lacking the EAEC probe sequence hybridised with either probe. These results clearly indicate that additional factors are involved in the AA phenotype in these EAEC strains.

Introduction

Enteroaggregative *Escherichia coli* (EAEC) is a category of diarrhoea-producing *E. coli* characterised by a distinct pattern of adhesion to epithelial cells whereby bacteria adhere to the cells and the intervening coverslip surfaces in 'stacked brick-like' aggregates [1]. EAEC have been implicated as agents of persistent diarrhoea among children in developing countries [2, 3] and recently have been associated with outbreaks of diarrhoea [4, 5]. However, the pathogenesis of EAEC diarrhoea remains unclear.

An EAEC DNA probe (CVD432), consisting of a segment from the 60-MDa plasmid of a prototype strain, has been used for the identification of EAEC strains [6]. However, lack of homology with this probe segment has been reported in some strains characterised as EAEC by the aggregative adherence (AA) phenotype [7].

Two fimbriae, designated aggregative adherence fimbria I and II (AAF/I and AAF/II), have been described in EAEC isolates [8, 9]. Both are plasmid-encoded and are required for expression of the AA phenotype. AAF/I is a bundle-forming fimbria which has been described in EAEC strain 17-2 [8]. The AAF/I-encoding genes are localised in two unlinked regions of a 60-MDa plasmid [8]. Region 1 encodes a cluster of structural and accessory biogenesis genes and region 2 encodes AggR, a transcriptional activator which is required for fimbria expression in strain 17-2. Based on mutational analysis and protein homology data, *aggA* has been shown to encode the major fimbrial subunit of AAF/I. AAF/II is genetically, phenotypically and morphologically distinct from AAF/I [9]. The fimbrial subunit gene of AAF/II has been identified and designated *aafA*. AAF/II confers adherence to human intestinal cells by EAEC strain 042 [9], which has been shown to elicit diarrhoea in human volunteers [10].

There are no data available describing the prevalence of AAF/I and AAF/II in a large number of EAEC isolates from a single epidemiological study. Therefore, the aim of this study was to evaluate the

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distribution of *aggA* and *aafA* genes among EAEC isolates from children with diarrhoea and age-matched controls in São Paulo, Brazil.

Materials and methods

EAEC isolates

For this study, 155 EAEC isolates, previously isolated and identified in a case-control study of 200 children with acute diarrhoea and 200 age-matched controls 1–4 years old were selected (TAT Gomes, unpublished observations). These children attended the emergency room of Hospital Infantil Menino Jesus, which is a municipal hospital that provides medical assistance to children of low socio-economic status in the city of São Paulo, Brazil. All isolates displayed AA in the HeLa cell adherence assay [11] and 78 of them reacted with the EAEC probe [6].

DNA hybridisation

aggA and *aafA* genes were detected by nucleotide probe hybridisation. The *aggA* probe was prepared by PCR with plasmid DNA extracted from strain 17-2 as template and the following oligonucleotides, as described by Savarino *et al.* [8]: SJS5-GCGTTAGAAA-GACCTCCAATA and SJS6-GCCGGATCCTTAAA AATTAATTCCGGC. PCR cycling conditions were: initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. These oligonucleotides amplify a product corresponding to the *aggA* gene of AAF/I. The *aafA* probe consisted of the 1.7-kb *EcoRI* fragment of pJC2 [9]. This fragment comprises *aafA* and part of an upstream gene encoding an AAF/II-specific fimbrial chaperone. DNA probe fragments were radiolabelled and used in colony blot assays as described previously [11].

Results

The results obtained in this study are summarised in Table 1. Only 21 (13.5%) of the 155 EAEC isolates hybridised with either *aggA* (9.7%) or *aafA* (3.9%)

Table 1. Distribution of *aggA* and *aafA* among EAEC faecal isolates from children 1–4 years old in São Paulo, Brazil

Adherence/reaction with EAEC probe	Total strains studied	Number (%) of strains reacting with	
		<i>aggA</i> *	<i>aafA</i> †
AA/+	78	15 (19.2)	6 (7.7)
AA/–	77	0	0
Total	155	15 (9.7)	6 (3.9)

AA, aggregative adherence to HeLa cells.

**aggA* probe, AAF/I structural subunit gene.

†*aafA* probe, AAF/II structural subunit gene.

probes and none with both. AAF/I and AAF/II genes were found exclusively among EAEC probe-positive isolates.

Of 78 isolates displaying AA and reacting with the EAEC probe (AA/+), 19.2% and 7.7% hybridised with the *aggA* and *aafA* probes, respectively. None of the 77 isolates displaying AA but lacking the EAEC probe sequence (AA/–) hybridised with either probe.

Discussion

The *aggA* and *aafA* genes were found in 9.7% and 3.9%, respectively, of 155 EAEC faecal isolates from children 1–4 years old in São Paulo, Brazil. Czczulin *et al.* [9] studied 51 EAEC strains by colony blot hybridisation and also found that AAF/I was more frequent than AAF/II. However, in that study the incidence of each gene sequence (*aggA* 31%, *aafA* 12%) among all strains was higher than that obtained in the present study. In this study none of the EAEC isolates that lacked homology with the EAEC probe reacted with either *aggA* or *aafA* probes; similar findings were reported by Czczulin *et al.* [9].

EAEC are apparently similar to enterotoxigenic *E. coli* (ETEC) and require a large number of different colonisation factors [7]. The prevalence of various colonisation factors of ETEC displays geographical variation, which may also be the case among EAEC.

Interestingly, all *aafA* positive isolates hybridised with the diffusely adherent *E. coli* (DAEC) probe (data not shown). This probe represents a part of the *daaC* gene, an accessory gene required for synthesis of the F1845 fimbria [12]. This finding suggests a genetic relatedness between the accessory genes of F1845 and AAF/II. This has been confirmed in sequence analysis of the AAF/II biogenesis loci (JP Nataro, unpublished observations).

These results clearly indicate that additional fimbrial or non-fimbrial adhesins, or both, may be involved in the AA phenotype in the population studied. Other studies have demonstrated by electron microscopy [7] the presence of several different fimbriae on the surface of EAEC strains, but only AAFs have been characterised at the genetic level [8, 9]. The role of AAF/I and AAF/II in diarrhoeal disease remains unclear. The involvement of AAF/II in intestinal colonisation has been suggested by the *aafA* mutant which no longer adheres to human intestine in tissue culture [9] and an AAF/II-producing strain that causes diarrhoea in volunteers [10]. Thus, the possibility exists that AAF/II-producing strains may be more virulent in man. Epidemiological studies should be conducted in other geographical areas to provide a broader epidemiological analysis of these adhesins in the pathogenesis of EAEC-associated diarrhoea.

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