

## Prevalence and Characteristics of the O122 Pathogenicity Island in Typical and Atypical Enteropathogenic *Escherichia coli* Strains<sup>∇</sup>

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**The presence of the pathogenicity island (PAI) O122 genes, *efa1* (*lifA*), *sen*, *pagC*, *nleB*, and *nleE*, in typical and atypical enteropathogenic *Escherichia coli* (EPEC) strains was investigated. The simultaneous occurrence of all genes was statistically associated with diarrhea due to atypical EPEC. Detection of the complete PAI O122 could aid in the identification of potential pathogenic strains of atypical EPEC.**

Enteropathogenic *Escherichia coli* (EPEC) and Shiga toxin (Stx)-producing *E. coli* (STEC) are important human enteropathogens (9). EPEC is further subgrouped into typical (tEPEC) and atypical (aEPEC) EPEC (8, 21). tEPEC strains are major causative agents of acute diarrhea in infants in developing countries, whereas aEPEC strains affect children and adults worldwide (5, 7, 9, 21). The main difference between tEPEC and aEPEC is the presence of the EPEC adherence factor (EAF) plasmid in tEPEC (8, 21). This plasmid encodes the bundle-forming pilus (BFP), which mediates localized adherence to intestinal cells (9), which is an essential property to differentiate tEPEC from aEPEC strains (1, 7, 21).

Formation of the attaching-and-effacing (AE) lesion is the major virulence mechanism of EPEC and an additional virulence property of enterohemorrhagic *E. coli* (EHEC) strains, a subset of STEC strains (9). This lesion consists of intimate bacterial adherence to the intestinal epithelial cells, cytoskeleton remodeling, and microvillus effacement (9). The genes encoding the AE lesion are located in a pathogenicity island (PAI) known as the locus of enterocyte effacement (LEE) (9). Despite the recognized importance of AE lesion formation, other putative virulence genes among AE-producing *E. coli* strains have been described.

Efa1 (EHEC factor for adherence) is an adhesin originally described in some EHEC strains (16). The *efa1* gene is almost identical to *lifA*, an EPEC gene encoding lymphostatin (LifA) (13). LifA inhibits the proliferation of mitogen-activated lymphocytes and the synthesis of proinflammatory cytokines (13). Efa1/LifA contributes to EPEC adherence to epithelial cells and is critical for intestinal colonization by *Citrobacter rodentium*, which is an AE lesion-producing bacterial pathogen of mice (12). Although, in the prototype EHEC O157:H7 strain EDL933, *efa1* (*lifA*) lacks the 3' region (*efaC*), the adherence ability of this strain is preserved (20).

*efa1* (*lifA*) is outside the LEE and inside PAI O122 (16). This island also harbors genes which are very similar to *pagC* of

*Salmonella enterica* serovar Typhimurium, *sen* of *Shigella flexneri*, and two *C. rodentium* non-LEE genes, *nleB* and *nleE*. *PagC* is required for bacterial survival within macrophages and is immunogenic to humans, while *sen* encodes an *S. flexneri* enterotoxin (10). *NleB* is linked to colonization and disease in mice (11), and *NleE* induces polymorphonuclear (PMN) trans-epithelial migration and is involved in the blockage of NF- $\kappa$ B activation (15, 24). Afset et al. (2) found that, of 182 virulence genes searched for among aEPEC strains, 12 were statistically associated with diarrhea, including *efa1* (*lifA*) (having the strongest association with the disease), *nleB*, and *nleE*.

In STEC the association of LEE, *efa1* (*lifA*), *sen*, and *pagC* was strongly correlated with virulence and disease severity (10). As the presence of PAI O122 genes have been searched for in only a limited number of tEPEC serotypes and in a few aEPEC strains (2, 4, 10, 14, 16), in this study we investigated the presence of *efa1* (*lifA*) and its location in PAI O122 as well as the presence of *sen*, *pagC*, *nleB*, and *nleE* among well-characterized tEPEC and aEPEC strains isolated from diarrheic and nondiarrheic patients.

A total of 152 strains (45 tEPEC and 107 aEPEC strains) of various serotypes were studied. These strains were isolated from diarrheic (114 strains) and nondiarrheic (38 strains) patients (5, 6, 22). Prototype tEPEC strain E2348/69 and EHEC strain EDL933 were used as positive controls, and *E. coli* HB101 was used as a negative control.

The presence of *efa1* (*lifA*) was screened by colony blot hybridization assays under stringent conditions (18). The *efa1* and *efa5'* DNA probes were fragments amplified from strain E2348/69 with primers described elsewhere (16) corresponding to both the internal and the 5' regions of the *efa1* (*lifA*) gene, respectively.

For better characterization of PAI O122 and the *efa1* (*lifA*) gene, PCR schemes and primers described previously were used (2, 3, 10, 14) to search for the presence of the *efa* 3' region, *sen*, *pagC*, *nleB*, and *nleE* using Mastermix (Promega, Madison, WI). Localization of *efa1* (*lifA*) in PAI O122 was investigated with Elongase (Invitrogen Life Technologies, Carlsbad, CA).

The *efa1* (*lifA*) gene was found in 60 (39.5%) out of 152 strains studied and was more prevalent among tEPEC strains

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TABLE 1. Serotypes and origin of tEPEC and aEPEC strains with complete<sup>a</sup> PAI O122

| Serotype (no. of strains)                 | Origin <sup>b</sup> |
|-------------------------------------------|---------------------|
| <b>tEPEC</b>                              |                     |
| O55:H <sup>-</sup> (1) <sup>c</sup> ..... | P                   |
| O55:H6 (4) .....                          | 3 P/1 C             |
| O111:H <sup>-</sup> (6) .....             | P                   |
| O111:H2 (6) .....                         | P                   |
| O127:H6 (1) .....                         | P                   |
| O142:H6 (2) .....                         | P                   |
| O145:H45 (1) .....                        | P                   |
| O127:H40 (1) <sup>c</sup> .....           | C                   |
| <b>aEPEC</b>                              |                     |
| O55:H7 (5) .....                          | 4 P/1 C             |
| O119:H2 (7) .....                         | P                   |
| O128:H2 (4) .....                         | P                   |
| O132:H8 (1) .....                         | C                   |
| NT:H8 (1) .....                           | P                   |
| NT:H34 (1) .....                          | P                   |

<sup>a</sup> Presence of all five genes (*efa* [*lifA*], *efaC*, *sen*, *pagC*, *nleB*, and *nleE*).

<sup>b</sup> P, patient; C, control.

<sup>c</sup> This strain did not present *efa* (*lifA*) in PAI O122, as detected by PCR schemes described in the text.

than aEPEC strains (62.2% versus 30.0%, respectively). Among all the strains analyzed only one (aEPEC) presented a truncated *efa1* (*lifA*) gene, like EHEC strain EDL933, as evidenced by lack of amplification with primers annealing to the 3' region (*efaC*) of *efa1* (*lifA*) (3) (Tables 1 and 2). This strain was isolated from a diarrheic patient and belonged to serotype O145:nonmotile, thus corroborating a previous finding of a truncated *efa1* (*lifA*) gene in an EHEC strain of the same serogroup (14).

In more than 95% of the *efa1* (*lifA*)-positive strains, this gene was located in PAI O122, as detected by the presence of the 6.5-kb region between *efa1* (*lifA*) and open reading frame (ORF) Z4326 (14) (Tables 1 and 2). Failure to detect *efa1* (*lifA*) in PAI O122 in five strains may indicate either that it is located in another spot on these bacterial genomes or that recombination events have occurred in their primers' annealing regions.

Although 92 strains (17 tEPEC and 75 aEPEC strains) were devoid of *efa1* (*lifA*), one of these strains carried only *nleB*, four carried only *sen*, three carried only *nleB* and *nleE*, six presented *sen*, *nleB*, and *nleE*, and only one carried *sen*, *pagC*, *nleB*, and

TABLE 2. Distribution and characteristics of incomplete PAI O122 and *efa1* (*lifA*) genes in tEPEC and aEPEC serotypes from patients and controls<sup>c</sup>

| Serotype (no. of strains) | No. of strains presenting:  |                 |                                                     |            |             |             |             | Origin <sup>a</sup> |
|---------------------------|-----------------------------|-----------------|-----------------------------------------------------|------------|-------------|-------------|-------------|---------------------|
|                           | <i>efa1</i> ( <i>lifA</i> ) | <i>efaC</i>     | Location of <i>efa1</i> ( <i>lifA</i> ) on PAI O122 | <i>sen</i> | <i>pagC</i> | <i>nleB</i> | <i>nleE</i> |                     |
| <b>tEPEC</b>              |                             |                 |                                                     |            |             |             |             |                     |
| O55:H6 (1)                | 1                           | 1               |                                                     |            |             | 1           | 1           | P                   |
| O88:H25 (4)               | 4                           | 4               | 4                                                   | 4          |             | 4           | 4           | P                   |
| O88:H25 (1)               | 1                           | 1               | 1                                                   | 1          |             | 1           | 6           | P                   |
| O127:H40 (1)              |                             | NT <sup>b</sup> | NT                                                  | 1          |             | 1           | 1           | C                   |
| <b>aEPEC</b>              |                             |                 |                                                     |            |             |             |             |                     |
| O11:H2 (1)                |                             | NT              | NT                                                  | 1          |             | 1           | 1           | P                   |
| O26:H <sup>-</sup> (4)    | 4                           | 4               | 4                                                   | 4          |             | 4           | 4           | 2 P/2 C             |
| O26:H <sup>-</sup> (1)    | 1                           | 1               |                                                     |            |             | 1           | 1           | P                   |
| O34:H <sup>-</sup> (1)    |                             | NT              | NT                                                  | 1          | 1           | 1           | 1           | P                   |
| O55:H7 (1)                | 1                           | 1               | 1                                                   | 1          | 1           | 1           | 1           | C                   |
| O55:H7 (1)                | 1                           | 1               | 1                                                   | 1          | 1           | 1           | 1           | P                   |
| O93:H <sup>-</sup> (1)    |                             | NT              | NT                                                  | 1          |             | 1           | 1           | P                   |
| O129:H11 (1)              |                             | NT              | NT                                                  |            |             | 1           | 1           | C                   |
| O132:H8 (1)               | 1                           | 1               | 1                                                   | 1          | 1           | 1           | 1           | P                   |
| O145:H <sup>-</sup> (1)   | 1                           | 1               | 1                                                   | 1          |             | 1           | 1           | P                   |
| O153:H7 (1)               | 1                           | 1               | 1                                                   | 1          |             | 1           | 1           | C                   |
| O157:H <sup>-</sup> (1)   |                             | NT              | NT                                                  |            |             | 1           | 1           | P                   |
| NT:H <sup>-</sup> (1)     |                             | NT              | NT                                                  | 1          |             |             |             | P                   |
| NT:H7 (1)                 |                             | NT              | NT                                                  | 1          |             | 1           | 1           | C                   |
| NT:H9 (1)                 |                             | NT              | NT                                                  |            |             | 1           |             | P                   |
| NT:H25 (1)                | 1                           | 1               | 1                                                   | 1          |             | 1           | 1           | P                   |
| NT:H33 (1)                |                             | NT              | NT                                                  | 1          |             |             |             | P                   |
| NT:H40 (1)                |                             | NT              | NT                                                  | 1          |             | 1           | 1           | P                   |
| NT:H40,43 (1)             |                             | NT              | NT                                                  | 1          |             | 1           | 1           | C                   |
| NT:H46 (1)                | 1                           | 1               |                                                     |            |             |             |             | P                   |
| R:H <sup>-</sup> (1)      | 1                           | 1               | 1                                                   | 1          |             | 1           | 1           | P                   |
| R:H28 (1)                 |                             | NT              | NT                                                  | 1          |             |             |             | P                   |
| R:H40 (1)                 |                             | NT              | NT                                                  | 1          |             |             |             | C                   |
| R:H40 (1)                 |                             | NT              | NT                                                  |            |             | 1           | 1           | P                   |

<sup>a</sup> P, patient; C, control.

<sup>b</sup> NT, not tested.

<sup>c</sup> The following serotypes showed none of the five PAI O122 genes tested (numbers of strains are in parentheses): tEPEC serotypes O55:H<sup>-</sup> (1), O86:H34 (2), O119:H6 (6), O142:H6 (2), O142:H34 (3), and O145:H45 (2) and aEPEC serotypes O2ab:H45 (1), O11:H16 (1), O98:H8 (1), O16:H<sup>-</sup> (1), O19:H<sup>-</sup> (1), O26:H<sup>-</sup> (1), O39:H<sup>-</sup> (1), O49:H<sup>-</sup> (1), O49:H10 (1), O51:H<sup>-</sup> (1), O63:H6 (2), O70:H2 (1), O85:H<sup>-</sup> (1), O101:H33 (2), O109:H9 (1), O111:H9 (2), O123:H19 (1), O124:H40 (1), O125:H6 (2), O145:H34 (1), O154:H9 (1), O157:H<sup>-</sup> (1), O157:H16 (2), O160:H19 (1), O162:H<sup>-</sup> (1), O177:H<sup>-</sup> (1), NT:H<sup>-</sup> (11), NT:H2 (1), NT:H8 (4), NT:H11 (1), NT:H11,21,34 (1), NT:H19 (2), NT:H29,31 (1), NT:H34 (2), NT:H38 (1), NT:H40,43 (1), NT:NT (2), R:H<sup>-</sup> (2), and R:H33 (1).

TABLE 3. Prevalence of PAI O122 in tEPEC and aEPEC strains isolated from patients and controls

| EPEC type | PAI O122 form <sup>a</sup><br>(no. of strains) | No. (%) of strains from: |           |
|-----------|------------------------------------------------|--------------------------|-----------|
|           |                                                | Patients                 | Controls  |
| tEPEC     | Complete (22)                                  | 20 (52.6)                | 2 (28.6)  |
|           | Incomplete (23)                                | 18 (47.4)                | 5 (71.4)  |
| Total     |                                                | 38 (100)                 | 7 (100)   |
| aEPEC     | Complete (19) <sup>b</sup>                     | 17 (22.4)                | 2 (6.5)   |
|           | Incomplete (88)                                | 59 (77.6)                | 29 (93.5) |
| Total     |                                                | 76 (100)                 | 31 (100)  |

<sup>a</sup> As examined for the presence of *efa1* (*lifA*), *sen*, *pagC*, *nleB*, and *nleE* genes. Complete, presence of all five genes; incomplete, one to four genes present.

<sup>b</sup>  $P = 0.04$  by Fisher's exact test.

*nleE* (Table 2). In other studies (2, 17) aEPEC strains carrying PAI O122 genes but lacking *efa1* (*lifA*) were also found.

Karmali et al. (10) demonstrated that the severity of disease caused by STEC and EHEC is due to the association of PAI genes. Another study describing PAI O122 genes in outbreak-associated non-O157:H7 STEC strains showed the existence of modules (different combinations of virulence genes) that determined the outcome of infection *in vivo* (23). In one of these modules, the simultaneous presence of *efa1* (*lifA*), *sen* (also named *ent*), *pagC*, and *nleB* provided a higher virulence potential (23). Unfortunately, in this study we could not associate different virulence degrees with PAI modules because the strains analyzed were isolated during epidemiological retrospective studies of acute diarrhea. Moreover, EPEC strains usually cause a smaller spectrum of virulence than EHEC.

A complete PAI O122 (carrying *efa1* [*lifA*], *pagC*, *sen*, *nleB*, and *nleE*) was found in 27.0% of the strains studied and was more prevalent in tEPEC than in aEPEC (48.9% versus 17.8%, respectively). The predominance of four PAI O122 genes in tEPEC was also observed by Scaletsky et al. (19). In the present study, occurrence of a complete PAI was observed in most tEPEC serotypes (Table 1) whereas incomplete PAIs were found only in tEPEC strains from serotypes O88:H25 and O127:H40. tEPEC O55:H6 strains carried either complete or incomplete PAIs (Tables 1 and 2).

Considering the strains in both EPEC groups altogether, the presence of a complete PAI was significantly associated with diarrhea ( $P = 0.005$  by Fisher's exact test). Significant association was also observed in the aEPEC group ( $P = 0.04$  by Fisher's exact test) (Table 3) but not in the tEPEC group ( $P = 0.22$  by Fisher's exact test), because of the higher number of tEPEC strains presenting complete PAI in controls.

This study shows that, although tEPEC and aEPEC strains may harbor complete and incomplete versions of PAI O122, a strong association between the presence of a complete PAI O122 and diarrhea was observed only in aEPEC. In fact, Bielaszewska et al. (4) reported that the presence of PAI O122 associated with LEE in aEPEC strains of the O26 serogroup would aid in their virulence potential. One can expect that the association of PAI O122 modules with LEE in aEPEC strains increases the pathogenicity of these strains circulating in our setting. Therefore, detection of complete PAI O122 could aid

in the identification of potentially pathogenic strains within this pathotype.

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