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# Plasmid Encoded Enterotoxin (Pet) Gene in Enteroaggregative *Escherichia coli* Isolated from Sporadic Diarrhea Cases

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Nataro et al. first reported that some *Escherichia coli* isolates from diarrhea patients had a capacity to adhere to HEp-2 cells and the surface of glass Petri dishes, and named them Enteroaggregative *E. coli* (EAggEC) (1). Its relation to persistent diarrhea in infants and HIV patients and to some diarrheas in adults has been indicated, though not conclusively (2).

The aggregative adherence of EAggEC was due to the presence of aggregative adherence fimbriae (AAF), AAF/I and AAF/II, whose expression is positively controlled by the *aggR* gene (2). This *aggR* gene is present in many EAggECs, but some *aggR*-positive *E. coli* isolates have no AAFs (3,4). EAggEC was found to produce EAggEC heat-stable enterotoxin (EAST1 encoded by *astA*, molecular weight 4.1 kDa) (2) and EAggEC plasmid encoded toxin (Pet encoded by *pet*, molecular weight 108 kDa) (5). Among them, EAST1 is widely distributed in pathogenic *E. coli* regardless of the aggregative adherence phenotype (2). Nataro et al. reported that two strains of EAST1-positive EAggEC produced different results when administered to volunteers (6); the difference between the two strains was later found to reside in the *pet* gene (5).

As the above data appeared to suggest that the *pet* gene was involved in the pathogenicity of EAggEC, and as no epidemiological investigation has been performed on the gene in Japan, we studied the incidence of the *pet* gene in *E. coli* isolated from diarrhea patients treated in a hospital in Aichi Prefecture from January 1989 to December 1992.

Among isolates from 9,684 patients (7,597 from the pediatric ward and 2,087 from the internal medicine ward), 364 isolates from different patients were aggregated using commercially available Enteropathogenic *E. coli* O Typing Sera (Denka Seiken, Tokyo) (7). The 364 isolates were screened for the presence of the *pet* gene by PCR using primers which were designed based on the *pet* sequence in GenBank accession No. AF056581. The sense primer was 5'-TTTCCAG CACTTCCTGTTCC-3', and the anti-sense primer was 5'-ATTTCCAACGTCTACGCCAT-3'. PCR amplification consisted of 30 cycles of incubation at 94°C for 60 sec, at 55°C for 45 sec, and at 72°C for 60 sec. The strain 042 (5) was used as a *pet* gene positive control. *Pet* negative controls were 17-2 and JM221 (O92:H33) strains showing aggregative adhesion (5,7), 886L (O111:H2) strain showing localized adhesion (7), and 251 (OUT:HUT) strain showing diffused adhesion (7). The genes of *aggR* and *astA* were detected using the method described by Moriya et al. (8).

Table. Detection of *pet* and *astA* genes in 65 *aggR* gene positive isolates

| | *pet* (+) | | *pet* (–) | |
|---|---|---|---|---|
| | serotypes | | serotypes | |
| *astA* (+) | O126:H27 | 12 | O111:H21 | 11 |
| | O126:HUT | 3 | O111:HUT | 1 |
| | | | O127a:H21 | 2 |
| | Total | 15 | Total | 14 |
| *astA* (–) | | | O86a:H27 | 24 |
| | | | O86a:H9 | 1 |
| | | | O86a:HUT | 2 |
| | | | O55:H10 | 7 |
| | | | O126:H19 | 2 |
| | Total | 0 | Total | 36 |
| Total | | 15 (23%) | | 50 (77%) |

The figures indicate number of isolates.

Among the 364 isolates which reacted to the Enteropathogenic *E. coli* O Typing Sera, 65 had the *aggR* gene. As shown in the Table, the *pet* gene was detected in 15 isolates (23%) among *aggR* positives. Eslava et al. reported that 15% of *E. coli* of the EAggEC phenotype isolated from diarrhea patients in six countries including Thailand, Peru, and the Philippines were *pet* gene-positive (5). Czeczulin et al. reported that 18% of EAggEC isolated from Thailand, Mexico, Peru and nine other countries were *pet* gene-positive and that, among *aggR*-positive EAggECs, 24% were *pet* gene-positive (3). Therefore, our data was quite comparable to those of previous reports.

All the 15 *pet*-positive isolates were *astA*-positive, and belonged to the O126 serotype. As Moriya et al. (8) reported that O126 *E. coli* isolated from healthy people possessed *aggR* and *astA*, it remains to be elucidated whether or not *pet* is directly associated with the pathogenicity of O126. Strains of serotypes of O33:H16, O44:H18, O111:H2, and OUT:H10

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isolated from the diarrhea of children have been reported to possess *pet* (3). These serotypes were not included in this study because none of them were isolated in this study. In order to clarify the role of *pet* in pathogenicity, further study will be required by examining the *pet* gene in a wide range of diarrhea patient isolates including the above serotypes.

In conclusion, 17.9% (65/364 isolates) of O sero-typable Enteropathogenic *E. coli* isolates from diarrhea patients had *aggR*, and 23% (15/65) of these *aggR*-positive *E. coli* had *pet* gene. This frequency was quite comparable to the previous studies performed in developing countries.

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