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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/1 and AAF/I, 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 entero-toxin (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 0 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'-TTTCCACGACTTCTGTCC-3', and the anti-sense primer was 5'-ATTTCCAACGTCTACGCCC-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 JM22 (092:H33) strains showing aggregative adhesion (5,7), 886L (0111: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).

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 0126 serotype. As Moriya et al. (8) reported that 0126 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 0126. Strains of serotypes of O33:H16, O44:H18, O111:H2, and OUT:H10...
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.

Strains O42 and 17-2 were kindly obtained from Dr. James Nataro, and JM221, 886L, and 251 from Dr. Blay Kay.

REFERENCES