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High-Pathogenicity Island of *Yersinia* spp. in *Escherichia coli* Strains Isolated from Diarrhea Patients in China

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The high-pathogenicity island (HPI) of *Yersinia* has been observed in 93% of 60 enteroadhesive *Escherichia coli* strains and 80% of *E. coli* strains isolated from blood samples. In the present study we investigated 671 fecal samples from patients with diarrhea in Shandong Province, China, and isolated HPI-harboring *E. coli* from 6.26% of the samples. The isolation rates for patients with diarrhea in three age groups, 10 to 20, 30 to 40, and 50 to 60 years, were 6.70, 12.35, and 10.81%, respectively. Therefore, HPI-harboring *E. coli* is the third most frequently isolated enteric pathogen from patients with diarrhea. Vomiting and abdominal pain were recorded for 33.33 and 66.67% of the patients, respectively. Stools with blood were observed for 9.52% of the patients. Twenty-four of 42 (57%) patients experienced a temperature over 37.4°C. These observations indicate that HPI-harboring *E. coli* is one of the major causes of diarrheal disease in China and that the clinical symptoms caused by HPI-harboring *E. coli* differ from those caused by enteroadhesive *E. coli*.

Yersinia pestis, Y. pseudotuberculosis serotype O1 to O3, and *Y. enterocolitica* biotype 1B strains possess a chromosomal determinant that has recently been designated the high-pathogenicity island (HPI) (3, 6, 7). In *Y. pestis*, the HPI comprises about 35 kb of chromosomal DNA linked to a 68-kb independent mobile pigmentation segment and includes the genes involved in iron storage and uptake, such as the *irp1* and *irp2* genes, which code for iron-repressible high-molecular-weight proteins HMWP1 and HMWP2, respectively, and the *fyuA* or *psn* gene (which code for ferric yersiniabactin uptake or pesticin sensitivity, respectively) (6, 8, 9, 10). The pathogenic strains of *Y. enterocolitica* biotype 1B carry a 45-kb stretch of chromosomal DNA comprising the *irp1-irp2* and *fyuA* genes without a pigmentation segment, which was shown to be important for *Y. pestis* to block the flea proventriculus (16, 24, 25).

Diarrheagenic *Escherichia coli* strains have been classified into several categories, such as enterotoxinogenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroadhesive *E. coli* (EAEC), and enterohemorrhagic *E. coli* (EHEC). The different categories can be identified on the basis of virulence factors (20). Schubert et al. (26) reported that 93% of 60 EAEC strains and 80% of *E. coli* strains isolated from blood samples hybridized with an *irp2* gene probe. The HPI was infrequently detected in strains of EPEC, ETEC, and EIEC. The gene for the HPI island has been proved to be absent from *Shigella* strains and *Salmonella enterica* serovars Enteritidis and Typhimurium (26). Recently, Karch et al. (18) reported that the HPI is present in 56 (27.2%) of 206 Shiga toxin-producing *E. coli* strains but is absent from all of 37 *E. coli* O157:H7 and *E. coli* O157:H− strains tested. Bach et al. (1) observed that, in addition to *E. coli*, the HPI is present in some species of *Citrobacter* and *Klebsiella*. In this study, we isolated HPI-harboring *E. coli* strains from patients with diarrhea and correlated the presence of HPI-harboring *E. coli* with clinical symptoms.

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A pilot study was carried out with 43 *E. coli* strains isolated from patients with diarrhea in Beijing, China, in 1987, 1988, and 1989. These strains did not belong to any recognized category of diarrheagenic *E. coli* strains, such as EPEC, EHEC, EIEC, ETEC, or EAEC, as demonstrated by DNA probe hybridization (28). PCR analysis showed that 34.9% (15 of 43) of these strains yielded *irp1*, *irp2*, and *fyuA* fragments identical in size to those of *Y. enterocolitica*. All the positive strains were further confirmed by colony blot hybridization with *irp1*, *irp2*, and *fyuA* genes amplified from *Y. enterocolitica* as probes. Two *E. coli* strains harboring *irp1*, *irp2*, and *fyuA* genes were subjected to Southern hybridization. The purified chromosomal DNAs from the two strains were digested with *Eco*RI and hybridized with *irp1*, *irp2*, and *fyuA* DNA probes (Fig. 1). For the *fyuA* gene, the Southern hybridization patterns of the two *E. coli* strains were identical to that of *Y. enterocolitica*. For the *irp1* and *irp2* genes, the molecular sizes of the hybridized DNA fragments were different from that of *Y. enterocolitica*. It seems that an *Eco*RI restriction site of the *irp1* genes was absent from *E. coli* strain (26).

For the PCR analysis, 30 cycles of denaturation (94°C, 1 min), extension, and annealing (at an annealing temperature [T_m] of 1 min) with one final extension step (72°C, 8 min) were performed. The sequences of the primers used for the PCRs (and the size of the amplified fragment [*S*], T_m, and the extension time [*E*] at 72°C) were as follows: (i) *irp1* forward primer (HPI 1), 5′-GGCGTCTCCTCCTTTGGTATT-3′; *irp1* reverse primer (HPI 2), 5′-GTGATTCCCGCTGTTGATGTT-3′ (*S*, 1,729 bp; T_m, 60°C; *E*, 2 min); (ii) *irp2* forward primer (HPI 3), 5′-GCGACGGGAAGCGATGAC-3′; *irp2* reverse primer (HPI 4), 5′-CGCAGTAGGCACGATGTTGTA-3′ (*S*, 287 bp; T_m, 62°C; *E*, 1 min); (iii) *fyuA* forward primer (HP5), 5′-GC GACGGGAAGCGATTTA-3′; *fyuA* reverse primer (HP6), 5′-CGCAGTAGGCACGATGTTGTA-3′ (*S*, 774 bp; T_m, 62°C; *E*, 1 min) (26). The primers were designed according to the published HPI DNA sequence (8, 9, 26). Strains of *Y. enterocolitica* WA (O8) and enteroaggregative *E. coli* O42 (EAggEC) were used as positive controls for the detection of HPI genes (2). For Southern blots, the restriction enzyme-digested genomic DNA fragments and PCR products were resolved through 0.8% agarose gels. The DNA was transferred from the

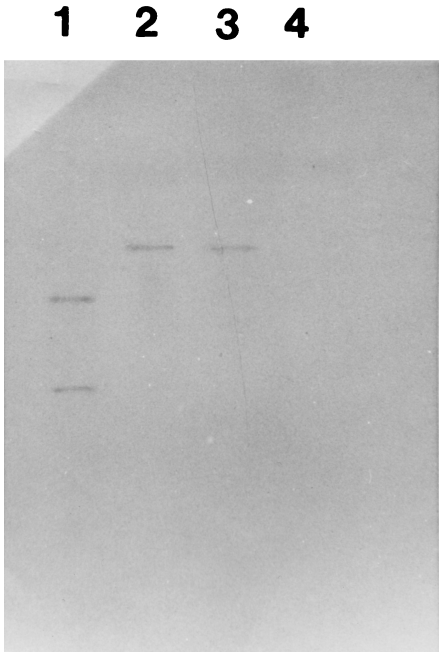

FIG. 1. Southern hybridization profile of the *Eco*RI-digested chromosomal DNA obtained with an *irp1*-specific probe. Lanes: 1, *Y. enterolitica* O8 WA (positive control); 2, *E. coli* F77 (*irp1*, *irp2*, and *fyuA* positive); 3, *E. coli* E1835 (*irp1*, *irp2*, and *fyuA* positive); 4, *E. coli* HB101 (negative control).

gel to Zeta-Probe BT blotting membranes (Bio-Rad Laboratories, Richmond, Calif.). After prehybridization at 68°C for 2 h and addition of heat-denatured probe, the blots were incubated overnight at 68°C. Digoxigenin labeling of the probes and hybridization were performed with a DNA labeling and detection kit (Boehringer, Mannheim, Germany) according to the manufacturer's instructions.

In order to reveal the frequency of isolation of HPI-harboring *E. coli* strains and the relationship of the HPI-harboring *E. coli* from patients with diarrhea and the associated clinical symptoms, the fecal samples from patients with diarrhea visiting the outpatient units of four appointed hospitals in Shandong Province were routinely collected and cultured for enteric bacterial pathogens in 1998; the patients had not received antibiotic therapy. The enteric bacterial pathogens for which screening was performed included *Vibrio cholerae*, *Vibrio parahaemolyticus*, *S. enterica* serovars Typhi and Typhimurium, *Shigella flexneri* 2a, *Shigella sonnei, Aeromonas* species, *Pseudomonas aeruginosa, Plesiomonas shigelloides,* EPEC, EIEC, and ETEC. When the patients visited the outpatient units, the clinical symptoms were examined and recorded on diarrhea disease investigation sheets by trained doctors. All of the data for patients with culture-confirmed cases of diarrhea caused by the organisms mentioned above were then collected and analyzed.

Among a collection of 671 fecal samples, 448 yielded recognized enteric pathogens, with *Shigella* species being the most frequently isolated pathogen, while 176 yielded pure but not recognized diarrheagenic *E. coli* strains in diagnostic antiserum kits. However, 42 strains of *E. coli* hybridized with DNA probes specific for the *irp2* gene, of which one strain also hybridized with the *ipaB* probe specific for EIEC. None of them hybridized with an EAggEC-specific DNA probe (2). By PCR analysis, the *irp2* and *fyuA* genes of HPI were confirmed to be present in these strains. The HPI has recently been reported to be present in as many as 90% of the EAEC strains tested (26). HPI-harboring *E. coli* has now identified in diarrheal patients of Shandong Province, China. The rate of isolation (6.26%) of HPI-harboring *E. coli* appears to be higher than those for ETEC, EIEC, or EPEC in this study. Therefore, it was the third most frequently isolated enteric bacterial pathogens studied, after *V. parahaemolyticus* and *Shigella* species (Table 1). It should be mentioned that the 42 isolates of HPI-harboring *E. coli* could not be considered EAggEC since they did not hybridize with the EAggEC-specific DNA probe (27). By HEp-2 cell adherence assay, many strains can be identified as EAggEC. However, some *E. coli* strains could adhere to HEp-2 cell in an aggregative pattern, but do not hybridize to the EAggEC-specific probe. Moreover, not all EAggEC isolates harbor the HPI (2, 20).

Nataro and Kaper (20) referred to EAEC as EAggEC. EAggEC does not secrete an enterotoxin such as the heat-labile or heat-stable toxin and has the ability to adhere to HEp-2 cells in an aggregative pattern (20). Baudry et al. (2) had developed a 1.0-kb plasmid-derived fragment as an EAggEC-specific diagnostic probe. In a prospective study of 513 Venezuelan infants with diarrhea and 241 age-matched controls, EAEC strains were found in 26.9% of diarrheal patients and 15% of control (11, 14). Several studies have suggested the association of EAEC with diarrhea in pediatric patients, especially those with persistent diarrhea (4, 5, 17, 20). With the data from outbreaks, sporadic cases, and the volunteer study, it has been suggested that EAEC causes a watery, mucoid, secretory diarrheal illness with low-grade fever and little to no vomiting (20). In volunteers infected with EAEC, the stools were generally mucoid and low volume without occult blood or fecal leukocytes. It appears that EAEC infection may be accompanied by a subtle form of mucosal inflammation (20).

In our study, HPI-harboring *E. coli* has been isolated from all age groups. It was isolated from 6.70% (14 of 209) of patients with diarrhea under age 10 years. The isolation rates

TABLE 1. Bacterial pathogens from patients with diarrhea in Shandong Province

Bacterial species	No. of strains isolated	Isolation rate (%)
Shigella flexneri 2a	161	23.99
Shigella sonnei	10	1.49
Vibrio parahaemolyticus	58	11.24
ETEC	26	3.87
EPEC	14	2.09
EIEC	19	2.83
HPI-harboring *Escherichia coli*	42	6.26
Atypical *Escherichia coli*	134	19.99
Salmonella enterica serovar Typhimurium	15	2.29
Yersinia enterocolitica	9	1.34
Pseudomonas aeruginosa	9	1.34
Citrobacter freundii	22	3.28
Proteus	22	3.28
Klebsiella	11	1.64
Enterobacter cloacae	13	1.94
Enterobacter aerogenes	1	0.25
Enterobacter agglomerans	2	0.30
Morganella morganii	4	0.60
Serratia liquefaciens	3	0.45
Providencia	6	0.89
Hafnia spp.	1	0.15
Total (exclusion of atypical *E. coli*)	448	66.8

TABLE 2. Clinical symptoms of 42 patients with diarrhea related to HPI-harboring *E. coli* and 134 patients with diarrhea related to atypical *E. coli*

Symptoms	No. (%) of patients with the symptom	
	HPI-harboring *E. coli*	Other atypical *E. coli*
Nausea	14 (33.33)	44 (32.8)
Vomiting	11 (26.19)	40 (29.9)
Diarrhea the following no. of times/day:		
3–5	9 (21.43)	26 (19.4)
6–8	16 (38.10)	36 (26.9)
9–10	12 (28.57)	48 (35.8)
>10	5 (11.90)	24 (17.9)
Form of feces		
Mucous	23 (54.76)	47 (35.1)
Watery	8 (19.05)	56 (41.8)
With blood	4 (9.52)	18 (13.4)
Unformed	7 (16.67)	12 (9.0)
Inappetence	26 (61.90)	54 (40.3)
Abdominal pain	28 (66.67)	40 (29.9)
Vapidity	21 (50)	41 (30.6)
Headache	10 (23.8)	23 (17.2)
Abdominal movement	8 (19.05)	14 (10.4)
Temp		
Normal	18 (42.86)	32 (23.9)
37.4–37.9°C	12 (28.57)	45 (33.6)
38–38.9°C	8 (19.05)	43 (32.1)
>39°C	4 (9.52)	14 (10.4)

for other age groups are as follows: 3.60% (4 of 111) for those ages 10 to 19 years, 4.43% (7 of 158) for those ages 20 to 29 years, 12.35% (10 of 81) for those ages 30 to 39 years, 3.85% (2 of 52) for those ages 40 to 49 years, 10.81% (4 of 37) for those ages 50 to 59 years, and 4.35% (1 of 23) for those >60 years of age. It seems that HPI-harboring *E. coli* can cause diarrhea in all age groups and is most frequently detected in association with diarrhea in children under age 10 years (14 of 44 bacteriologically confirmed cases). Among the patients with HPI-harboring *E. coli*-related diarrhea, vomiting was observed in 33.33% of the patients; a normal temperature, low-grade fever, and high-grade fever were observed in 40.48, 28.57, and 28.57% of the patients, respectively. A total of 66.67% of the patients experienced abdominal pain; mucoid, watery, and liquid green stools were observed in 54.76, 19.05, and 11.90% of the patients, respectively. Stools with blood were observed in 9.52% of the patients. In the patients with diarrhea related to atypical *E. coli* isolates, 35.1 and 41.8% of the patients had watery and mucoid feces, respectively; 89.1% of the patients experienced a low, medium, or high temperature (Table 2). Only 23.9% (13 of 134) of the patients had a normal body temperature. These observations suggested that the diarrhea related to HPI-harboring *E. coli* is a watery, mucoid illness (31 of 42 patients [73.81%]) with no fever or a low-grade fever (30 of 42 patients [71.43%]). A total of 78.57% (33 of 42) patients had diarrhea more than six times a day (Table 2). The remarkable differences observed between the clinical symptoms related to HPI-harboring *E. coli* and those related to atypical *E. coli* are stool forms and body temperature.

The gene products of the *fyuA-irp* gene cluster of the HPI island may benefit *E. coli* pathotypes (22, 25). The yersiniabactin has a possible cytotoxic effect on T cells (12). Pyochelin, the yersiniabactin-like siderophore of *P. aeruginosa*, could promote damage to endothelial cells by formation of free radicals (21, 26). The *irp2* gene product, HMWP2, has extensive similarity to a superfamily of adenylate-forming enzymes involved in the nonribosomal peptide synthesis of not only siderophores but also peptide antibiotics (19, 23, 24, 26). By analogy with yersiniae, it has been suggested that the *fyuA-irp* gene cluster may contribute to the virulence of certain pathogenic *E. coli* strains, such as EAEC and septicemia-causing *E. coli* strains (26). Since the reason for the *Yersinia* HPI among EAEC strains is unknown, more studies are needed to clarify its clinical importance and the pathogenic role that it plays.

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