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**Hafnia alvei** in Stool Specimens from Patients with Diarrhea and Healthy Controls

JOUKO RIDELL,1* ANJA SIITONEN,2 LARS PAULIN,3 LEENA MATTILA,2 HANNU KORKEALA,1 AND M. JOHN ALBERT4

Department of Food and Environmental Hygiene, University of Veterinary Medicine,1 Laboratory of Enteric Pathogens, National Public Health Institute,2 and Institute of Biotechnology, University of Helsinki,3 Helsinki, Finland, and International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh4

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We found an epidemiological association of *Hafnia alvei* with diarrhea, because the organism was isolated from 12 of 77 (16%) adult Finnish tourists to Morocco who developed diarrhea and from 0 of 321 tourists without diarrhea (*P < 0.001*). From another group of 112 adult Finnish diarrheal patients, only 2 (2%) yielded *H. alvei*. In contrast to some Bangladeshi strains of *H. alvei*, the Finnish strains were negative for the attachment-effacement lesion by an in vitro fluorescent actin staining test and also did not show homology to the *Escherichia coli* attachment-effacement gene (eaeA) by PCR. These results suggest that a mechanism or mechanisms other than the attachment-effacement lesion may also be involved in the association of *H. alvei* with diarrhea.

Recently, *Hafnia alvei* strains isolated from diarrheal feces of Bangladeshi children have been reported to cause attaching-effacing (AE) lesions typical of enteropathogenic *Escherichia coli* (EPEC) (2, 5). These strains also hybridized with the EPEC eaeA probe. The eaeA gene is the only virulence-associated factor described so far for *H. alvei*. Genes belonging to the eaeA gene cluster are reported to occur also in enterohemorrhagic *E. coli* and in one biotype of *Citrobacter freundii* (9). The aim of the present study was to clarify the potential role of *H. alvei* as an enteropathogen. We determined the isolation rate of *H. alvei* in subjects with and without diarrheal symptoms and screened the isolates for the ability to produce the AE lesion and for the possession of the eaeA gene.

Fecal samples were obtained from three groups of adult Finnish people (510 people in all). Diarrhea group 1 consisted of 77 travelers examined during or immediately after travel to Morocco between January and February 1989. Diarrhea group 2 consisted of 112 patients examined between March and June 1993 as a routine diagnostic measure when they consulted a physician because of diarrhea symptoms. Of these patients, 71 had recently traveled abroad, mostly to the Mediterranean or Far Eastern countries. The third group formed a control group of 321 nondiarrheal travelers examined immediately after their return from Morocco in 1989. All stool samples were cultured by standard methods and studied for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Aeromonas*, and *Plesiomonas* species as described previously (6). The specimens of diarrhea group 1 and the control group were also processed for different types of diarrheagenic *E. coli*, rotaviruses and adenoviruses, and intestinal parasites (8). In diarrhea group 1 and the control group, one to three colonies were subcultured from primary nonselective Drigalski-Conradi agar for species identification with the API 20 E system (BioMerieux, Marcy l’Etoile, France). In diarrhea group 2, the cultures on nonselective cystine-lactose-electrolyte-deficient agar were screened for the presence of *H. alvei*: 5 to 10 colonies were tested for the hydrolysis of L-proline-p-nitroanilide for detecting *H. alvei* (3). The final species identification was performed with the API 20 E system. Statistical analysis was done with the χ² test.

The study also included six *H. alvei* strains isolated from diarrheal patients in Bangladesh and previously reported to produce AE lesions (strains 10457, 10790, 19982, 38/90, 9194, and 12502 [2]). Strains 10790 and 19982 were also reported to hybridize with the EPEC E2348/69-specific eaeA probe. EPEC E2348/69 (127:H6) obtained from J. P. Nataro (Center for Vaccine Development, University of Maryland, School of Medicine, Baltimore) was used as a positive control when searching for the eaeA gene. In addition, *H. alvei* ATCC 13337 (type strain) and ATCC 29927 (DNA reference strain) were included. The ability to produce the AE lesion was screened by the fluorescent actin staining test with HEp-2 cells (2). Possession of the eaeA gene was tested by PCR. For this determination, bacterial strains were grown in brain heart infusion broth (Difco, Detroit, Mich.) overnight at 37°C. Each sample was prepared by boiling 100 μl of a 10⁻³ dilution of bacterial culture (final bacterial concentration, 10⁸/ml) with 100 μl of 2% Triton X-100 for 10 min. Primers (sense, 5’GATCTCTGAAAGCGGATTTAAGC3’; antisense, 5’TCTCGGTGCAA TCCGCTTTAGGC3’) were derived from the conserved region (residues 2182 to 3372) of the EPEC E2348/69 (GenBank accession numbers M58154 and M34051) eaeA gene. Amplification were performed with a DNA thermal cycler (PTC-100; MJ Research, Watertown, Mass.) for 35 cycles of 30 s at 95°C, 30 s at 62°C, and 2 min at 72°C with 3 min of initial denaturation at 95°C and 5 min of final extension at 72°C in a 25-μl volume containing 5 μl of sample and 1 μl of Dynazyme DNA polymerase (Finnzymes, Espoo, Finland) in a buffer provided by the manufacturer and overlaid with 30 μl of sterile paraffin oil. Negative (*Serratia liquefaciens*) and positive (EPEC E2348/69) controls were included with each experiment. The amplification product generated in the PCR assay with *H. alvei* reference strain 10457 was cloned into *Salmonella* enterica serotype Typhimurium ΔmalC::Bluescript KS plasmid (Stratagene, La Jolla, Calif.). Two plasmids containing the insert were sequenced by the dyeoxy method with the AutoRead kit (Pharmacia, Uppsala, Sweden).
TABLE 1. Isolation of *H. alvei* from stool samples of diarrheal and nondiarrheal subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Diarrheal patients (n = 112)</th>
<th>Nondiarrheal subjects (n = 321)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1* (n = 77)</td>
<td>Group 2* (n = 111)</td>
</tr>
<tr>
<td>All subjects with <em>H. alvei</em></td>
<td>12 (16)*</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Subjects with <em>H. alvei</em> alone</td>
<td>4 (5)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Subjects with <em>H. alvei</em> together with other enteropathogens(s)</td>
<td>8 (10)*</td>
<td>0</td>
</tr>
<tr>
<td>Subjects with known enteropathogens</td>
<td>48 (62)*</td>
<td>12 (11)*</td>
</tr>
</tbody>
</table>

* Finnish travelers to Morocco in 1989 with or without diarrhea (partly described previously [6]).

* P < 0.001 versus diarrheal group 2 and nondiarrheal subjects.

* C. jejuni, five patients; *Aeromonas* spp., three patients.

* See reference 6.

Salmonella enterica, six patients; C. jejuni, four patients; *Aeromonas* spp., two patients; *Shigella sonnei*, one patient. In one sample, two pathogens were isolated.

logical association with diarrhea for this organism. It can be argued that *H. alvei* cells are transient organisms; if so, they should also have been isolated from controls without diarrhea. Since controls did not yield this organism, this possibility is unlikely. In positive specimens, we have found 2 to 10 colonies of *H. alvei*, and this organism was found significantly more frequently in patients with diarrhea acquired in Morocco than in diarrheal patients in Finland. It may be that *H. alvei* is more prevalent in Morocco than in Finland.

The present study confirmed that some Bangladeshi *H. alvei* strains previously reported to be positive for gene sequences homologous to eaeA were indeed so (2). However, strains isolated from Finnish subjects did not contain such a gene sequence. The absence of the eaeA gene sequence was further confirmed by a negative fluorescent actin staining test. It is possible that these strains may possess other virulence factors for production of diarrhea. The demonstration of such virulence factors and other case-control studies are needed to further argue for a role for *H. alvei* in causing diarrhea. In addition, the taxonomy of this species needs to be studied more closely. According to DNA-relatedness studies, biochemically defined species of *H. alvei* may consist of several genospecies (4, 10).

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REFERENCES


