ASSOCIATION OF VIRULENCE FACTOR-POSITIVE AND -NEGATIVE ENTEROAGGREGATIVE ESCHERICHIA COLI AND OCCURRENCE OF CLINICAL ILLNESS IN TRAVELERS FROM THE UNITED STATES TO MEXICO

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Abstract. The objective of the study was to determine if the presence or absence of virulence factor-positive and -negative enteroaggregative Escherichia coli (EAEC) determined the occurrence of illness or sub-clinical EAEC infection in travelers from the United States to Mexico. Sixty-five newly arrived college students from the United States submitted weekly stool samples for a four-week period of time. Among EAEC-infected subjects, diarrhea occurred in those with a defined virulence factor with the following frequency: aggA, 5 of 15 (33%); aggR, 3 of 11 (27%); aafA, 3 of 8 (38%); and aspU, 1 of 6 (17%). Twenty-two of 31 students (71%) had two or more EAEC infections. After the initial EAEC infection, only 4 (11%) of 31 students had a subsequent symptomatic EAEC infection. Our study suggests that clinical illness by EAEC is not explained by presence of a defined EAEC virulence factors, and we provide suggestive evidence that EAEC infection protects against future symptomatic infection.

INTRODUCTION

Enteroaggregative Escherichia coli (EAEC) has been implicated as a cause of persistent diarrhea in children1 and acquired immunodeficiency syndrome–associated diarrhea,2,3 as well as acute diarrhea in travelers.4,5 Not all EAEC strains have been shown to cause diarrhea in humans. The EAEC are a heterogeneous group of bacteria that display a wide array of virulence factors. The relative contribution to disease for each one of these virulence factors is unclear. Furthermore, the interaction between the host immune response and heterogeneity of virulence factors of EAEC is complex.1,6,7 This prospective study examined the association of virulence factor-positive and -negative EAEC with the occurrence of clinical illness in travelers to Mexico.

MATERIAL AND METHODS

Sixty-five college students from the United States (age range = 18–36 years old) attending classes in Guadalajara, Mexico in July and August 1999 were offered the opportunity to participate in the study. Inclusion criteria included enrollment in the study and provision of a stool sample for study within 72 hours of arrival in Mexico and agreement to provide subsequent stool samples at the end of the first, second, and third weeks of their time in Guadalajara. Exclusion criteria included travel to a developing region of the world within three months or receipt of an antimicrobial agent with expected activity against bacterial enteric pathogens (e.g., trimethoprim-sulfamethoxazole or a fluoroquinolone) within one week prior to enrollment. The four stool samples per subject were submitted to our field laboratory and processed for enteric pathogens. Symptomatic infection or diarrhea was defined as passage of three or more unformed stools in a 24-hour period plus one or more signs or symptoms of enteric illness (i.e., nausea, vomiting, abdominal pain or cramping, fecal urgency, or dysentery). Participation in this study was voluntary and required the written informed consent of subjects prior to enrollment. The study was reviewed and approved by the Committee for the Protection of Human Subjects of the University of Texas-Houston Health Science Center and by the University of San Diego whose program the students attended in Guadalajara, Mexico.

All stool samples were tested for conventional enteric pathogens as described previously.7

Five E. coli-like colonies per stool sample retrieved from MacConkey agar plates were transported in individual peptone stabs to Houston. These E. coli-like colonies were then taken to the Center for Infectious Diseases at the University of Texas-Houston for further analyses that included detection of toxin genes of ETEC6 and the HEp-2 cell adherence assay to detect the patterns of adherence. The presence of EAEC was determined by the HEp-2 cell adherence assay.6 Briefly, a chamber slide (Dynatek, Naperville, IL) was seeded with HEp-2 cells that had been grown at 37°C in 5% CO2 in minimal essential medium (MEM) (Gibco-BRL, Gaithersburg, MD) supplemented with 10% fetal calf serum. Escherichia coli to be tested were grown overnight statically at 37°C in tryptic soy broth (BBL Microbiology Systems, Cockeysville, MD) with 1% D-mannose. The cell culture medium in the chamber slide was then replaced with MEM containing 1% D-mannose without antibiotics and E. coli were added before incubation at 37°C for three hours. The slide was washed three times with phosphate-buffered saline, fixed with 100% methanol, and stained with crystal violet (Difco, Detroit, MI). Escherichia coli strains H10407, 042, and HS were used as quality controls. Each E. coli strain was tested twice in a blinded fashion. A sample was interpreted as positive for EAEC if it showed the characteristic “stacked-brick” aggregative appearance.6

The EAEC virulence factors were studied in each isolated EAEC by a PCR. Oligonucleotide primers for EAEC virulence factors aggA, aggR, aafA, and aspU were selected based on previous studies.1,6,7 An amplification mix of 98 μL of PCR mixture (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl2, 100 μM each of dATP, dCTP, dGTP, and dTTP, and 2.5 units of AmpliTaq polymerase [Perkin-Elmer, Norwalk, CT]), 25 pmol of each primer, and 2 μL of DNA template were heated for five minutes at 94°C. The reaction mixture was then subjected to 35 cycles (94°C for 30 seconds, 50°C for one minute, and 72°C for 45 seconds) and then to a final extension at 72°C for seven minutes in a DNA thermal cycler (Perkin-Elmer). Ten microliters of the amplified PCR product was then sub-
ected to electrophoresis on a 1% agarose gel with a 1-kb DNA molecular weight marker (Gibco-BRL). A positive result was determined by the presence of a PCR product of expected size.

The statistical function used to determine significant differences was the chi-square test.

RESULTS

Of the 65 students enrolled in this study, 31 (48%) students had at least one stool sample positive for an EAEC during the four-week study. Sixty-five stool samples were provided at week one, 62 at week two, 55 at week three, and 55 at week four. In the 31 students who had at least one EAEC isolated from their stools, 31 stool samples were provided at week one, 30 at week two, 28 at week three, and 25 at week four. Eighteen (58%) of the 31 students had an asymptomatic infection and 13 (42%) students had a symptomatic EAEC infection.

Including both asymptomatic and symptomatic infections, 22 (71%) of 31 students had two or more EAEC infections. Nine students (29%) had one sample positive for EAEC, 13 (42%) students had two, six (19%) had three, and three (10%) had four weekly stool samples positive for EAEC.

The number of symptomatic EAEC infection by virulence factor and by week of study is shown in Table 1. AggA (37.5%) and aggR (27.5%) were the two most commonly isolated virulence factors. Fifteen EAEC isolates had the virulence factor aggA. Of those 15, five (33.3%) had a symptomatic EAEC infection. Eleven EAEC isolates had an EAEC isolated with the virulence factor aggR, and three (27.3%) of those 11 students developed diarrheal illness. Eight (20%) students had an EAEC isolated positive for the virulence factor aafA and three experienced diarrhea (37.5%). Six (15%) of the students were positive with the virulence factor aspU and one (16.7%) developed diarrhea.

The number of initial and subsequent EAEC infection in the various students is shown in Figure 1. Nine (29%) of the 31 students had an initial symptomatic infection, and 22 (71%) of the students had an initial asymptomatic EAEC infection. After the initial EAEC infection, four (13%) students had a subsequent symptomatic EAEC illness (P = 0.12, 95% confidence interval = 0.78–9.62), and 27 students (87%) developed asymptomatic EAEC reinfection.

DISCUSSION

The present study failed to show an association between virulence factor-positive EAEC and the occurrence of clinical illness in travelers from the United States to Mexico. Virulence factor-positive EAEC occurred in asymptomatic students, and virulence factor-negative EAEC occurred in symptomatic students. One student had an asymptomatic EAEC infection that carried all four virulence factors: aggA, aggR, aafA, and aspU. These results suggest that host genetic and environmental factors may be more important in influencing the disease outcome of EAEC infection. Also, additional virulence properties of EAEC strains may help to explain human virulence for this emerging pathogen.

The most common virulence factor isolated from EAEC was aggA (37.5%). AggR and aafA were commonly found, seen in 27.5% and 20% of EAEC strains isolated, respectively. AspU was the least commonly found virulence factor, found in 15% of tested strains. Other studies have also shown that aggA and aggR are common EAEC virulence factors. Most EAEC strains possess a 60–65-mD plasmid, designated pAA (aggregative adherence), which encodes several virulence factors such as the AA fimbria, AAF/I, and AAF/II. The AAF/I genes include aggA, which encodes the major fimbrial subunit. The AAF/II genes include aafA, which has been shown to mediate adherence to the intestinal mucosa.

Table 1

<table>
<thead>
<tr>
<th>Virulence factors</th>
<th>Week of study after arrival in Mexico</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>aggA</td>
<td>1/3 (33.3)</td>
<td>2/3 (66.7)</td>
<td>0/5</td>
<td>2/4 (50)</td>
<td>5/15 (33.3)</td>
<td></td>
</tr>
<tr>
<td>aggR</td>
<td>0/1</td>
<td>2/2 (100)</td>
<td>0/5</td>
<td>1/3 (33.3)</td>
<td>3/11 (27.3)</td>
<td></td>
</tr>
<tr>
<td>aspU</td>
<td>0/1</td>
<td>0</td>
<td>0/4</td>
<td>1/1 (100)</td>
<td>1/6 (16.7)</td>
<td></td>
</tr>
<tr>
<td>aafA</td>
<td>0/1</td>
<td>2/2 (100)</td>
<td>0/2</td>
<td>1/3 (33.3)</td>
<td>3/8 (37.5)</td>
<td></td>
</tr>
</tbody>
</table>

Although not statistically significant, our data suggest that...
early symptomatic or asymptomatic infection with EAECl is associated with protection against subsequent symptomatic EAEC infection. Twenty-two (71%) of 31 EAEC infections occurred one or two weeks after arrival in Mexico. Regardless of the presence of symptomatic or asymptomatic EAEC infection, there was an 85–91% chance of not developing subsequent EAEC diarrhea once infection had occurred. These results suggest that the early EAEC infection “primes” the immune system of the host by providing protection against future symptomatic EAEC infections regardless of presence of one of virulence properties studied. Immunity does not appear to develop to asymptomatic EAEC infection because many of the students developed multiple EAEC infections with isolated strains possessing different virulence factors. No fecal IgA was measured in this study. However, it is likely that this immunoglobulin is involved in the protection of subsequent EAEC infection. This is an area of future interest in our laboratory.

All EAEC isolates were identified by the HEp-2 cell assay. The HEp-2 assay remains the “gold standard” for identification of EAEC. We believe that this assay is accurate and that presence of bacterial clusters in a stacked-brick configuration provides definitive identification of EAEC strains. No analysis of the pAA plasmid was performed in this study. It may, however, be of interest to determine the genotype correlates associated with phenotype. Other diagnostic tools of EAEC are currently being evaluated. A DNA probe based on a 1.0-kb DNA fragment labeled PCVD432 from the 60-md EAEC strains lack definable virulence properties making genotype correlates with phenotype difficult.

An additional area of interest is the possibility of plasmid loss upon transport. It is a largely unstudied area, and there is no existing evidence that loss occurs with EAEC.

In conclusion, there was no association of presence or absence of one or more of four defined virulence factors in EAEC infection and the occurrence of clinical illness. Many students had multiple EAEC infections during their four-week stay in Mexico. Early infection with EAEC appears to be associated with protection against subsequent symptomatic EAEC infection. Our study suggests that the spectrum of disease is likely determined by both the presence of pathogen-specific virulence factors and by the ability of the host to respond to the inflammatory stimulus caused by EAEC.

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