SHORT REPORT: CHARACTERIZATION OF ENTEROAGGREGATIVE ESCHERICHIA COLI ISOLATES FROM IRANIAN CHILDREN

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Abstract. We have previously shown that enteroaggregative Escherichia coli (EAEC) is an important pathogen among Iranian infants and children. To better understand the characteristics of EAEC in Iran, we analyzed EAEC isolates for the presence of pAA plasmid-borne factors. Ninety-eight E. coli strains that displayed the aggregative adherence (AA) pattern on HeLa cells were hybridized with the CVD432 (AA) probe and with genes encoding enteroaggregative heat-stable enterotoxin-1 and aggregative adherence fimbriae (AAF) I and II. Our data suggest that AAF/II is common in this population and that AAF/I and AAF/II can sometimes be detected in the same E. coli isolate. Surprisingly, we have found that AA probe-negative strains in Iran share virulence factors with AA probe-positive isolates and therefore may be more similar to probe-positive strains than previously believed.

Enteroaggregative Escherichia coli (EAEC) has been associated with persistent and acute diarrhea in developing countries and in Europe. Most EAEC strains harbor a 60 to 65 MDa virulence plasmid (pAA), from which Baudry and others derived empirically a DNA fragment probe (called the aggregative adherence [AA] or CVD432 probe); the probe has been widely used for epidemiologic studies. The pAA plasmid also encodes aggregative adherence fimbriae (AAF/I and II) and the toxin enteraggregative heat-stable enterotoxin-1 (EAST-1). Notably, neither the AA probe nor any specific virulence factor is found in all EAEC strains, suggesting that EAEC strains may be highly heterogeneous.

To assess the genetic characteristics of EAEC from Iranian children, we studied strains isolated from a previously described study. DNA was extracted by means of the alkaline lysis method and dotted to nitrocellulose. Hybridization was performed under stringent conditions by the digoxigenin labeling and detection system (Boehringer Mannheim, Indianapolis, IN). DNA probes included the 0.7-kb EcoRI/I fragment of pAA probe; the 125-bp KpnI/XmnI fragment of pSS125 (EAST-1); the 350-bp EcoRI/PstI fragment from clone pJC2 (for AAF/I); and a 1.7-kb EcoRI fragment from clone pC2 (for AAF/II). HeLa adherence was tested by the method described by Nataro and others.

Of the 98 strains exhibiting the aggregative HeLa cell adherence pattern, 46 (46.9%) reacted with the AA probe. Hybridization with the EAST-1 probe revealed that only 38 (38.8%) of EAEC isolates harbored the gene encoding EAST-1; 23 (60.5%) of EAST-1–positive strains were AA probe-positive and 15 (39.5%) were AA probe-negative (P ≤ 0.05). It has been shown that EAST-1 is not restricted to EAEC isolates; nonetheless, our data suggest a difference between probe-positive and probe-negative strains with regard to the frequency of this gene (Table 1). AAF/I fimbrial genes were detected in 8 (8%) of the EAEC strains; all of these were AA probe positive (for a prevalence of 17% within the probe-positive group). AAF/II, in contrast, was detected in 25% of all strains, comprising 41% of AA probe-positive strains and, notably, 12% of probe-negative strains. The simultaneous presence of genes encoding AAF/II and AAF/II was detected in 5 strains, all probe-positive isolates.

Our data reinforce the heterogeneity of EAEC with regard to both toxins and adherence factors. Early studies of EAEC suggested that most strains were AA probe positive. More recent studies have detected a low prevalence of AA probe-positive strains, and the relationship of these strains (if any) to AA probe-negative strains is unclear. However, phylogenetic studies have suggested that some AA probe-negative EAEC strains may nevertheless carry the pAA plasmid and EAEC chromosomal factors and therefore may lack only the AA probe-homologous sequences. This is a plausible hypothesis because the AA probe was derived empirically and does not encode a known virulence factor. Our data support this conjecture in that we found that 12% of probe-negative strains carried AAF/II genes.

However, we also note that the overall prevalence of AAF/I and AAF/II in EAEC worldwide cannot be estimated from these studies. Recent publications support not only EAEC heterogeneity, but also significant geographic variation in the frequencies of virulence factor genes.

EAEC is an emerging pathogen, but its mechanisms of pathogenicity are only partially understood. This fact is complicated by strain heterogeneity. Further studies in Iran and elsewhere are needed to clarify this complicated picture.

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