Escherichia coli Associated with Childhood Diarrheas

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We studied 2,246 episodes of childhood diarrhea over a 2-year period in a general hospital serving a population of about one million on the island of Hong Kong. Rotavirus (24%) and nontyphoid salmonellas (23%) were the most common causal agents, followed by Campylobacter sp. (9%). Rotavirus occurred largely during winter, whereas salmonellas and Campylobacter sp. occurred more commonly during summer and autumn, respectively. Enterotoxigenic Escherichia coli was rarely isolated (1%), and the isolates were clonally diverse. A small percentage (2.8%) of E. coli had serological specificities commonly associated with enteropathogenic E. coli, but only two of the isolates were also positive for HEP-2 adhesive factor. Pure or heavy and predominant growth of E. coli was obtained in repeated stool cultures of 432 (19%) of these episodes, which did not yield any of the above-mentioned pathogens. Although associated with diarrhea, these E. coli isolates possess neither the pathogenic attributes of enterotoxigenic E. coli nor the properties commonly associated with enteropathogenic E. coli. Enteroinvasive E. coli and enterohemorrhagic E. coli were considered unlikely causes on clinical grounds.

Infected diarrhea is a common childhood disease, the etiology of which changes according to geography, climatic conditions, nutritional status, and other circumstances. Previous studies showed that for urban centers rotavirus and Salmonella sp. are the important causal agents (5, 13, 21), whereas in rural areas, especially where water supply and public health measures are poor, enterotoxigenic Escherichia coli (ETEC), Shigella sp., and other bacterial agents assume an important role (3). We describe the results of a 2-year study of childhood diarrhea in patients 5 years old or younger who were admitted since 1983 to a hospital which serves a population of about one million on the island of Hong Kong. The pattern of diarrheal disease in this population is similar to that of other urban centers, with rotavirus being the most important of the conventional intestinal pathogens (21). The study also shows that E. coli is associated with a large proportion of the childhood diarrhea episodes in this city. The nature of this association was further investigated by serogrouping (9) and by testing the isolates for the presence of enterotoxins (7, 14, 17, 18), colonization factors (10, 11), and enteroadhesive factor (6, 19). Only a small proportion of these E. coli isolates possessed the above-mentioned pathogenic attributes. Most of the isolates, if they are indeed the causes of the diarrheal episodes, appear to cause diarrhea by unknown mechanisms.

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MATERIALS AND METHODS

Strains. E. coli strains isolated from 2,246 infants and children less than 5 years old with diarrhea in Queen Mary Hospital of Hong Kong were collected from May 1983 to October 1985. Stool specimens from these patients were examined for Shigella, Salmonella, Vibrio, and Campylobacter spp. by standard methods (4, 12, 13, 16) and for rotavirus, as previously described (2), by enzyme-linked immunosorbent assay. Four lactose-fermenting colonies identified as E. coli by standard biochemical tests, were selected at random for ETEC screening as described previously (23). E. coli isolates collected from May 1983 to May 1984 were concurrently evaluated by the Biken (14), infant mouse (7), and DNA filter (17, 18) assays. Other strains were subjected to DNA filter assay only, and ETEC isolates detected were confirmed by the Biken and infant mouse assays. Enteropathogenic E. coli (EPEC) serogrouping, as described by Edwards and Ewing (9), was performed on all E. coli strains with Burroughs Wellcome E. coli antiserum. EPEC isolates identified by serogrouping were subjected to the HEP-2 cell adhesion test by the biological and DNA filter assays (6, 19). E. coli strains from 100 children suffering from nondiarrheal diseases were collected as a control group for ETEC and EPEC screening. In addition, E. coli isolates from stools of 15 neonates suffering from a diarrhea outbreak were collected in September 1982. Bacterial strains harboring recombinant plasmids EWD299 (LT), PRIT10036 (ST-P), and pSLM004 (ST-H) were supplied by S. Falkow of Stanford University. A bacterial strain carrying pMAR2 (enteroadhesive factor) and human enteroadhesive E. coli 2348 and B170 were provided by James Kaper of the University of Maryland. Standard human enteric E. coli strains H10407 (LT, ST-P, ST-H), and M421C1 (ST-H), were provided by S. Moseley of the Children’s Orthopedic and Medical Center, Seattle, Wash. A nontoxigenic E. coli strain, JP995, which was cured of all plasmids, was given to us by J. Ling of the Chinese University of Hong Kong.

DNA analysis. Plasmid DNAs from strains confirmed to be ETEC were extracted by the method of Kado and Liu (15), electrophoresed in horizontal agarose gels, transferred to nitrocellulose by the method of Southern (20), and hybridized with 32P-labeled DNA probes as described previously (23). E. coli plasmids of known molecular mass, including 40R660 (25.9 megadaltons [MDa]), 40R646 (37.8 MDa), 34R193 (31.7 MDa), 40R268 (64 MDa), 40R448 (77.6 MDa), and 28R823 (140.7 MDa), were electrophoresed in parallel with plasmid DNAs from the strains being studied.

Detection of CFA/I and CFA/II. Bacterial growth of ETEC obtained from 18-h-old Casamino Acids-yeast extract-salt agar cultures was subjected to hemagglutination with human group A, bovine, guinea pig, and chicken erythrocytes suspended in phosphate-buffered saline containing 1% man-
TABLE 1. Distribution of enteric pathogens in Hong Kong between May 1983 and October 1985

<table>
<thead>
<tr>
<th>Enteric pathogen</th>
<th>No. of cases (%)</th>
</tr>
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<tbody>
<tr>
<td>Rotavirus</td>
<td>536 (24)</td>
</tr>
<tr>
<td><em>E. coli</em> ETEC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21 (0.9)</td>
</tr>
<tr>
<td><em>E. coli</em> EPEC serogroups&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63 (2.9)</td>
</tr>
<tr>
<td>Others&lt;sup&gt;b&lt;/sup&gt;</td>
<td>432 (19)</td>
</tr>
<tr>
<td><em>Salmonella</em> sp.</td>
<td>510 (23)</td>
</tr>
<tr>
<td>Unknown</td>
<td>402 (18)</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>195 (9)</td>
</tr>
<tr>
<td>Mixed pathogens&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58 (3)</td>
</tr>
<tr>
<td><em>Shigella</em> sp.</td>
<td>29 (1)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Eight of the ETEC isolates were also positive for EPEC serogrouping.

<sup>b</sup> *E. coli* was isolated as predominant or pure growth from diarrhea patients from whom no other conventional pathogens were isolated. The nature of its association with diarrhea is unknown.

<sup>c</sup> Mixed pathogens included 33 rotavirus-*Salmonella* sp. infections, 11 rotavirus-*Campylobacter* sp. infections, 13 *Salmonella-Campylobacter* sp. infections, and 1 rotavirus-*Shigella* sp. infection.

nose to determine colonization factors I and II (CFA/I and CFA/II) (10, 11).

Detection of HEP-2 cell adhesive factor. All *E. coli* strains belonging to the classical EPEC by serogrouping (9) were examined for enteroadhesive factor on HEP-2 cells by biological (6) and DNA filter (19) assays which have previously been described.

Antibiotic resistance pattern. ETEC isolates were tested for antibiotic resistance by the disk diffusion method (1). Chloramphenicol, ampicillin, streptomycin, tetracycline, and sulfamethoxazole were tested.

Biotyping. ETEC isolates were subjected to sugar fermentation tests, as described by Edwards and Ewing, for identification of members of the family Enterobacteriaceae (9). The carbohydrates used for fermentation tests included adonitol, cellobiose, dulcitol, raffinose, rhamnose, salicin, sucrose, and xylose.

RESULTS

Between May 1983 and October 1985, we investigated 2,246 episodes of diarrhea among children 5 years of age or younger admitted to Queen Mary Hospital. The distribution of enteric pathogens found is shown in Table 1. Rotavirus was the most common pathogen, being isolated from 24% of the patients. Characteristically, the infection showed a marked seasonal distribution, occurring most frequently during winter, although sporadic cases also occurred during the other seasons (21). *Salmonella* sp. was the most common of the conventional bacterial pathogens, accounting for 23% of all the isolates. The infection was more common during summer but persisted throughout the year. There were over 20 different serotypes of *Salmonella* sp. (excluding *Salmonella typhi*) isolated during this period, with *S. typhimurium* being the most common of the isolates. Hong Kong depends on imports for most of its supply of meat and dairy products, and this may contribute to the large variety of *Salmonella* species isolated. There were 195 cases (9%) of *Campylobacter jejuni* detected, and the infection was more frequent
during autumn. The infection has become more prevalent in recent years and is believed to be related to the local poultry industry and its intense production method. Shigellosis was rare (1%); there were only 29 cases due to *Shigella flexneri* or *S. sonnei*, whereas the other species of *Shigella* were not isolated.

Repeated stool cultures excluded the above-mentioned pathogens as the probable causes of the remaining 918 diarrheal episodes. Of the latter, 402 (18%) patients yielded scant-to-moderate mixed growth of intestinal flora. Presumably, the disease was due to noninfectious causes or infection with other enteric viruses. The other 516 of these patients, however, yielded heavy and predominant or pure growth of *E. coli* on repeated stool cultures, with no other conventional intestinal pathogens being isolated from them. None of these 516 patients had symptoms resembling bacillary dysentery or hemorrhagic colitis.

To characterize the nature of *E. coli*-associated diarrhea in Hong Kong, we examined *E. coli* isolates from the 516 diarrheal patients and 100 control patients without diarrhea for the presence of ETEC and serogroups commonly associated with EPEC (EPEC serogroups). ETEC isolates were further tested for CFA/I and CFA/II, and the EPEC serogroups and eight strains of ST-H ETEC were also tested for HEP-2 adhesion as described in Materials and Methods. There were 21 ETEC isolates, and 8 were positive for ST-H and CFA/I (Table 2). All of these ST-H producers were of serogroup O126. Three isolates were positive for LT, ST-H, and CFA/II. The remaining isolates were positive for one or more enterotoxins, but they did not exhibit other pathogenic attributes. Apart from the eight strains of ST-H ETEC, there were 63 isolates of EPEC serogroups, 2 of which were also positive for HEP-2 adhesion. Identification of the above-mentioned pathogenic attributes in *E. coli* isolates of diarrhea patients was considered significant because they were not detected in isolates obtained concurrently from 100 patients from the same hospital with other diseases.

During the study period, several *E. coli* outbreaks occurred in other hospitals. On one such occasion, 17 of the 21 neonates aged 4 to 9 days in a maternity hospital developed watery diarrhea within 3 days after the appearance of the index case. ETEC was isolated from 15 of these patients. All of these isolates were identified as ST-H ETEC, with O126 serological specificity and CFA/I. Therefore, this outbreak strain may be clonally related to the ST-H ETEC strains

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>Enterotoxin</th>
<th>CFA/I</th>
<th>CFA/II</th>
<th>EPEC serogroups&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HEP-2 adhesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>8 (ST-H)</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4 (LT, ST-H)</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>1 (LT, ST-H)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>8 (LT)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>63</td>
<td>0</td>
<td>0</td>
<td>63</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>432</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>100 (nondiarrhea)</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> EPEC serogroups refer to the following O factor specificities which are commonly associated with EPEC: O18c, O26, O44, O55, O86, O111, O112, O114, O119, O124, O125, O126, O127, O128, and O142.

<sup>b</sup> ND, Not done.
different antibiotic resistance patterns. The enterotoxin plasmids in these isolates were simultaneously probed with ST-H and LT. All five strains harbored three different plasmids each (Fig. 3A). The blots of individual isolates were separately probed with both LT and ST, and the plasmids thus identified by either probe from each isolate were of similar sizes, except for one. In the latter, the LT plasmid had a larger molecular mass (65 MDa) than the ST-H plasmid (59 MDa) (data not shown). These results are supported by Fig. 3B, for which the blots were probed for LT and ST-H simultaneously. The LT and ST-H plasmids from one isolate (lane D) were clearly resolved, whereas the enterotoxin genes appeared to be encoded by the same or similar plasmids in the other four isolates (lanes A, B, C, and E).

**DISCUSSION**

The above-described pattern of childhood diarrhea for Hong Kong is akin to that in other major urban centers. Crowded living and possibly climatic conditions are probably major contributing factors to rotavirus being the most important of the causal agents. This is followed by food-borne infections due to nontyphoidal salmonellas and, to a lesser extent, *C. jejuni*. The virtually exclusive dependence of Hong Kong on imports for its food supplies is believed to have resulted in the large variety of *Salmonella* species circulating in this community. Effective public health measures and a good water supply, on the other hand, have reduced waterborne infection to a minimum in this city. Consequently, ETEC, which was primarily a waterborne agent (22), was encountered extremely rarely in the present study.

The rare ETEC isolates were clonally diverse. The most prevalent of these isolates was a strain designated ST-H O126, which caused both sporadic diarrhea and hospital outbreaks. These isolates carried an ST-H gene borne on a plasmid of 64 MDa. They were positive for CFA/I, had O126

isolated from sporadic cases at Queen Mary Hospital (Table 2). This contention was supported by the findings that all of these isolates, referred to as ST-H O126, had the same antibiotic resistance pattern (susceptible to ampicillin and resistant to chloramphenicol, sulfamethoxazole, streptomycin, and tetracycline) and that they also had the same biotype (fermentation of raffinose, rhamnose, salicin, sucrose, and xylose only). These isolates harbored one or more plasmids (Fig. 1). On Southern blotting, the ST-H gene of ST-H O126 was localized to a plasmid of 64 MDa (Fig. 1, lanes F to L). This was contrasted with ST-H ETEC isolates from Japan (lanes A to D). In these isolates, the ST-H gene was localized to plasmids of sizes between 50 and 76 MDa.

The isolates elaborating LT were as prevalent as those which elaborated ST-H. Unlike the latter, the LT ETEC isolates appeared to be clonally diverse. They exhibited different antibiotic resistance patterns and were of different biotypes. The LT was also localized to plasmids of sizes between 49 and 74 MDa (Fig. 2, lanes A to E).

FIG. 1. Identification of the ST-H plasmid in ETEC isolates from different regions. Plasmids of ETEC isolates from Japan (lanes A to D) (22), Thailand (lane E), sporadic cases (lanes F and I to L), and an outbreak of ETEC diarrhea (lanes G and H) in Hong Kong were visualized with ethidium bromide (A). Panel B shows blots of the same plasmids probed with ST-H.

There were five isolates which harbored two or more enterotoxin genes simultaneously. Three of these isolates (LT and ST-H) were positive for CFA/I but exhibited

FIG. 2. Identification of LT plasmids in ETEC isolates from Hong Kong and Thailand. Plasmids of ETEC from Hong Kong which were positive for LT (lanes A to E), LT, ST-H, and ST-P (lane F), or LT and ST-H (lanes G, H, and J) or of an isolate from Thailand which was positive for LT and ST-H (lane I) were visualized with ethidium bromide (A). The blots of these plasmids were probed with LT (B).
serological specificity, and exhibited the same antibiogram and biotype. The strain had persisted for, and was isolated throughout, the 2-year period of study. The above-mentioned properties and epidemiological features distinguish ST-H O126 from the other clonally diverse ETEC strains isolated sporadically during the same period. The latter isolates carry one or more enterotoxin genes borne on plasmids of various sizes and exhibit different antibiograms and biotypes. This disease pattern is not unexpected for an urban center such as Hong Kong, which has a good water supply and receives a large number of travelers, with many from neighboring regions where ETEC diarrhea is endemic (3).

The above-mentioned enteric pathogens accounted for only 63% of the diarrhea episodes presently investigated, and this concurs with the results of similar studies in other urban centers. With these pathogens excluded as the most probable causes of these episodes, repeated stool cultures from 402 patients (18%) yielded scant or moderate growth of mixed intestinal flora. Presumably these were diseases of a noninfectious nature or were caused by other enteric viruses or parasites. For the remaining 432 (19%) of these patients, E. coli was isolated as heavy and predominant or pure growth on repeated stool culture.

Further study of E. coli as a diarrheal agent depends on an understanding of the pathogenesis of the disease due to this group of agents. As indicated earlier, plasmidborne pathogenic attributes such as enterotoxins or CFA accounted for only a very small proportion of the episodes studied. Serogrouping identified from some of these patients certain strains of E. coli (3%) which are commonly associated with diarrhea caused by EPEC. The latter probably makes up a heterogeneous population of organisms having different pathogenic attributes (8). HEP-2 cell adhesive factor is believed to be one pathogenic attribute associated with some EPEC (8). However, only two of the present isolates with the serological specificity of EPEC were positive for HEP-2 cell adhesive factor. Most of the E. coli isolates possessed neither the pathogenic attributes presently investigated nor the serological specificities commonly associated with EPEC. Although not specifically investigated for, enteroinvasive and enterohemorrhagic E. coli strains are considered to be unlikely causes of the present diarrheal episodes on clinical grounds and because of the atypical phenotypes of enteroinvasive E. coli strains (22).

The large preponderance of E. coli isolates associated with childhood diarrhea but possessing none of the commonly described pathogenic attributes thus highlights the need for further research on the pathogenesis of childhood diarrhea associated with these organisms.

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