Enteroaggregative Escherichia coli Serotype O126:H27, Israel

Gila Shazberg,* Moshe Wolk,†1
Herbert Schmidt,‡ Iancu Sechter,†
Giora Gottesman,§ and Dan Miron¶

Enteroaggregative Escherichia coli (EAEC) is a newly diarrheagenic agent wherein several predominant serotypes are reported. We studied the association between those serotypes, as clonal indicators, and the trait of enteroaggregative adherence to host cells, tested by polymerase chain reaction. We also evaluated the clinical manifestations of infection in 17 hospitalized children by our most common EAEC serotype, O126:H27.

**The Study**

Clinical signs and the laboratory findings were evaluated for 17 children <2 years of age, hospitalized in four pediatric wards in different areas of Israel. All these children had gastroenteritis attributable to EAEC or enterotoxigenic *E. coli* (ETEC) of serotype O126:H27 (Table 1).

Serotyping was performed (10). To determine O-antigen, cultures were heated to 120°C for 1 h, then checked for agglutination with specific O-antiserum at 50°C overnight. For determination of H-antigen, motile cultures were grown overnight in nutrient broth, treated with 0.5% formaldehyde, and investigated for agglutination with specific H-sera at 50°C for 2 h.

To detect EAEC, we used PCR primers specific for a short sequence of the plasmid pAA of EAEC, which is necessary for adherence. Analysis for the presence of pCVD432 sequences was performed at the Institute of Hygiene and Microbiology, the University of Wuerzburg, and the Institute of Medical Microbiology and Hygiene, Technical University of Dresden, Germany. Briefly, *E. coli* were isolates grown overnight on L-agar, and a single colony was suspended in 50 µL of phosphate-buffered saline (PBS). Amplification was carried out in a total volume of 50 µL containing each nucleotide triphosphate at 200 µM, 30 pmol of each primer, 5 µL of 10-fold concentrated AmpliTaq DNA polymerase synthesis buffer, 1.5 mM MgCl2, 2.5 U AmpliTaq DNA polymerase (Applied Biosystems Applera, Weiterstadt, Germany), and 5 µL of template Oligonucleotides pCVD432/start (5′-CTG GCC AAA GAC TGT ATC AT-3′) and pCVD432/stop (5′-AAT GTA TAG AAA TCC GCC GT-3′) were purchased from Sigma-ARK GmbH (Darmstadt, Germany) (5). The PCR protocol comprises 30 rounds of amplification, each consisting of 30 s at 94°C, 60 s at 52°C, and 60 s at 72°C. The first cycle was preceded by a denaturation step of 10 min at 94°C, and the last extension cycle was followed by a final extension step of 10 min at 72°C.

Enterotoxins were determined by the Asialoganglioside-GM1 enzyme-linked immunosorbent assay (GM1-ELISA) method using the direct plate cultures technique. Heat-labile toxin (LT) was determined by GM1-ELISA using monoclonal antibodies against LT (11). Heat-stable toxin (ST) was determined in parallel in the same cultures by an inhibition-GM1-ELISA that used monoclonal anti-ST (12). The test was performed in two 96-well polystyrene microplates A&B (Nunc A/S Roskilde, Denmark) and comprises several steps. The plates were coated with GM1 by adding 0.1 mL of 0.3 nmol GM1 (Sigma, Rehovot, Israel) in 0.1 M PBS, pH=7.2, to each well. After the plates incubated overnight at room temperature, they were washed three times with PBS, blocked with 0.1% bovine

*Dr. Shazberg and Wolk contributed equally to this paper.

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1. Drs. Shazberg and Wolk contributed equally to this paper.
Finally washed once with PBS. To each of the GM1-coated serum albumin (BSA) in PBS for 30 min at 37°C, and then washed three times with PBS-Tween. Substrate was pre-
dissolved 10 mg of ortophenylene diamine in 10 mL of 0.1 M sodium citrate buffer (pH=4.5) to which 4 µL of 30% H₂O₂ was added. To each well in plates A and B, 0.1 mL of this substrate solution was added. After 20 min, the plates were read at 450 nm in a Micro ELISA Auto Reader spectrophotometer (Dynatech Inc., Alexandria, VA).

When the optical density (OD) decreased >50% as compared with the OD of anti-ST mixed with ST negative control culture, which run in parallel to the experimental wells, the result was considered ST positive. When the OD value at 450 was ≥0.100 above the background, the result was considered LT positive. Since serotype O126:H27 was prevalent in our EAEC cultures, we tried to isolate bacteriophages specific to the EAEC of this serotype from sewage water. Five unrelated strains of EAEC serotype O126:H27 were used. One milliliter of an early logarithmic broth culture of each strain was seeded in a bottle of 50-mL nutrient broth. After incubation of 3 h at 37°C, 5 mL of sewage water was added to each bottle. After a new incubation of 6 h, cultures were killed by adding 1 mL of chloroform, followed by intensive shaking. The next day the supernatant of each bottle was tested for activity on the respective strain. The isolated phages were then diluted and purified twice by single plaque isolation (13). The five phages were active on EAEC strains of serotype O126:H27.

From July 1999 to December 2001, we collected and characterized 1,368 isolates of diarrheagenic E. coli. Of these isolates, 88 (6.4%) belonged to one of the five most common EAEC serotypes, i.e., serotype O126:H27 (n=48), O111:H21 (n=16), O125 (n=11), O44:H18 (n=11), O7:H10 (n=2) (Table 2). The percentages of EAEC

### Table 1. Bacteriologic parameters and clinical signs of children with *Escherichia coli* O126:H27

| No. | Age (mo) | EAEC-PCR Phage sensitivity | ST | Diarrhea (days) | Dehydration | IV fluid | Vomit | Fever | Concomitant clinical findings |
|-----|----------|---------------------------|----|----------------|-------------|----------|-------|-------|-------------------------------|
| 1   | 6        | +                         | -  | + (12)         | +           | +        | +     | + 39 | Malabsorption, prolonged diarrhea |
| 2   | 1.5      | +                         | -  | + (40)         | +           | +        | -     | -    |                               |
| 3   | 5        | +                         | +  | + (4)          | +           | +        | +     | + 40 |                               |
| 4   | 2        | +                         | +  | + (4)          | +           | +        | +     | -    |                               |
| 5   | 15       | +                         | -  | + (2)          | +           | +        | +     | + 40 |                               |
| 6   | 21       | +                         | +  | + (4)          | +           | +        | +     | + 39 | Tonsilitis                     |
| 7   | 18       | +                         | +  | + (1)          | -           | -        | -     | -    |                               |
| 8   | 16       | +                         | +  | + (9)          | +           | +        | -     | + 39 | Otitis                         |
| 9   | 11       | +                         | -  | + (8)          | +           | +        | -     | + 40 | Tonsilitis                     |
| 10  | 16       | +                         | +  | + (9)          | +           | +        | +     | + 40 |                               |
| 11  | 15       | +                         | +  | + (7)          | -           | -        | +     | + 39 | UTI                            |
| 12  | 1        | +                         | +  | + (2)          | +           | +        | -     | -    |                               |
| 13  | 15       | +                         | +  | + (6)          | -           | -        | +     | + 40 | Tonsilitis leukocytosis        |
| 14  | 1.5      | NT                        | +  | + (5)          | -           | +        | -     | + 38.7 | Meningitis                     |
| 15  | 6        | NT                        | +  | + (1)          | +           | +        | +     | + 40 |                               |
| 16  | 1 week   | -                         | +  | + (6)          | +           | +        | -     | -    |                               |
| 17  | 9        | -                         | +  | + (5)          | +           | +        | +     | + 40 |                               |

*EAEC, enteraggregative *Escherichia coli*; PCR, polymerase chain reaction; ST, heat-stable toxin; UTI, urinary tract infections; NT, not tested.
However, we were not able to associate that serotype
has been reported as a common cause of diarrhea in chil-
be a pathogenic agent of young children who require hos-
Conclusions
Five patients (nos. 1, 2, 8, 9, 10) had prolonged diarrhea of
patients (nos. 12–17), while LT was not produced in any.
Of 12 non-EAEC cultures (Table 3). The sensitivity of this
our EAEC and non–EAEC cultures of this serotype. Four
E. coli O126:H27 was found in stools from 17 children
in four pediatric wards in various areas in Israel (Table 1).
The stools were watery; no mucus or blood was seen. Most
the children were dehydrated and needed IV treatment
with fluids and electrolytes. Some children vomited sever-
of serotype O126:H27 suggests that we found a clone that
might therefore belong to the pathotype of ETEC.
However, ST was apparently not the main diarrheagenic factor, since in the five children with prolonged
diarrhea no ST was produced. Some other kind of toxin
probably involved in these cases. In strains from some
patients (Table 1, numbers 12–15) we found both traits of
EAEC and ETEC in the same organism. A simple test to
identify EAEC in routine laboratory work is needed. A
possible solution is to use a phage sensitivity test in addi-
tion to serotyping, such as we used here for EAEC
O126:H27. Preliminary results suggest that this test (Table
is a reliable indicator. If this fact is confirmed on a large
number of strains, specific phages might also be selected
for EAEC of other serotypes.
The obvious accumulation of pCVD432-positive E. coli
of serotype O126:H27 suggests that we found a clone that
spread in Israel and probably has a selective advantage.

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Dr. Shazberg is a senior physician in a pediatric department.
Her research interest is pediatric infectious diseases.

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Table 2. Serotypes of enteroaggregative Escherichia coli (EAEC) evaluated by polymerase chain reaction

| E. coli serotype | O126:H27 | O111:H21 | O125:H?x | O44:H18 | O7:H10 | Totals |
|-----------------|----------|----------|----------|----------|--------|--------|
| No. positive    | 35 (73%) | 12 (75%) | H9=5     | H49=1    | 2 (18%)| 56     |
| No. negative    | 13 (27%) | 4 (25%)  | H49=3    | H6=2     | 9 (82%)| 32     |
| Totals          | 48       | 16       | 11       | 11       | 2      | 88     |

PCR–positive strains (Table 2) were as follows: 73% in E. coli O126:H27 and 75% in E. coli O111:H21. In E. coli O125, the percentage was approximately 50%, and in E. coli O44:H18, unlike reported elsewhere (14), this percentage was low.

To determine if the isolated phages were specific for the enteroaggregative strains of serotype O126:H27, the five phages were tested by spot test at routine test dilution on our EAEC and non–EAEC cultures of this serotype. Four phages were active on both kinds of strains. Only phage no. 4 was active on 33 of the 34 EAEC cultures and on 1 of 12 non-EAEC cultures (Table 3). The sensitivity of this phage was 97%, and its specificity was 91%. This phage could therefore be used as an indicator for AA in this E. coli serotype.

E. coli O126: H27 was found in stools from 17 children in four pediatric wards in various areas in Israel (Table 1). The stools were watery; no mucus or blood was seen. Most of the children were dehydrated and needed IV treatment with fluids and electrolytes. Some children vomited several times. All 17 patients had a normal leukocyte count for age. Twelve of them had high fever (38.7°C–40°C). Three of these 12 children had diarrhea concomitant with other diseases (patients 11, 13, and 14). Stool cultures of these three children were taken as part of an investigation of febrile disease. The same three children received antibiotic treatment; all others recovered without antibiotics. The length of hospitalization was 2–8 days. The duration of diarrhea was 1–40 days (median 5 days) starting, in some cases, before hospitalization. ST was produced in six patients (nos. 12–17), while LT was not produced in any. Five patients (nos. 1, 2, 8, 9, 10) had prolonged diarrhea of >1 week, characteristic of EAEC (15).

Conclusions
In our patients, EAEC serotype O126:H27 appears to be a pathogenic agent of young children who require hospitalization and dehydration treatment. This same serotype has been reported as a common cause of diarrhea in children from England (16), Japan (17), and Bangladesh (9). However, we were not able to associate that serotype exclusively with the enteroaggregative pathotype, since nonaggregative Ec O126:H27 strains from hospitalized children (patients 16 and 17 in Table 1) produced ST and might therefore belong to the pathotype of ETEC.

Table 3. Phage sensitivity of Escherichia coli O126:H27 compared to EAEC-PCR

| Phage sensitivitya | EAEC-PCR positive | EAEC-PCR negative |
|--------------------|------------------|------------------|
| Positive           | 33 isolates      | 1 isolate        |
| Negative           | 1 isolate        | 11 isolates      |

4EAEC PCR, enteroaggregative Escherichia coli polymerase chain reaction.
5Sensitivity = 97%; specificity = 91%.

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Address for correspondence: Moshe Wolk, Central Laboratories, Israel Ministry of Health, 9 Yaakov Eliav St., POB 34410, Jerusalem 91342, Israel; fax: 009722651828; email: Moshe.Wolk@eliav.heal.gov.il