Escherichia coli Contains Plasmids Coding for Heat-Stable b, Other Enterotoxins, and Antibiotic Resistance

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Plasmid DNAs obtained from 18 Escherichia coli isolates that hybridized with the heat-stable b (ST-b) enterotoxin gene probe were examined by Southern blot analysis for genes coding for heat-labile, ST-a, and ST-b enterotoxins with specific radiolabeled DNA probes. Four E. coli isolates contained plasmids coding for both heat-labile and ST-b enterotoxins, and one isolate contained a plasmid coding for ST-a and ST-b. Five of 11 isolates of antibiotic-resistant enterotoxigenic E. coli isolates containing ST-b-coding DNA transferred a plasmid coding for both antibiotic resistance and ST-b to E. coli K-12, suggesting that the widespread use of antibiotics could increase the distribution of genes coding for ST-b.

Enterotoxigenic Escherichia coli (ETEC) is an important cause of diarrhea in humans and livestock (3, 12, 18, 19, 24). ETEC strains produce a heat-labile toxin, LT (6, 23), and two distinctly different forms of heat-stable toxin, ST-a (7) and ST-b (5). E. coli that produce ST-a cause diarrhea in humans and animals and are detected by testing sterile culture supernatants in a sucking mouse assay (7). E. coli that produce ST-b cause water secretion in ligated piglet intestinal loops (5) but are negative in the infant mouse assay (7). What role ST-b-producing ETEC has in either human or animal diarrhea has not been determined due, in large part, to the cumbersome animal assay (19) required to identify ST-b.

The genes coding for ST-b have been cloned and sequenced (15, 22) and shown to be distinct from two different DNA sequences coding for ST-a (14, 21). A 460-base-pair DNA fragment coding for ST-b has been used as a probe to determine the incidence of E. coli containing genes coding for ST-b (ST-b E. coli) at pig farms (9) as well as in villages in northeastern Thailand (10). In a longitudinal study of two villages, five (22%) of 23 specimens contained E. coli that hybridized with a DNA probe for genes coding for ST-b, hybridized with genes coding for LT or ST-a, and produced either LT or ST-a (7, 23). In this study, plasmids isolated from E. coli from these five specimens, as well as E. coli that hybridized with only the ST-b gene probe from 13 other specimens, were examined by Southern blot analysis (28) to determine whether plasmids coding for ST-b were homogeneous in size, and whether genes coding for different E. coli enterotoxins were located on the same plasmid. ST-b E. coli were tested for antibiotic susceptibility, and antibiotic-resistant E. coli were examined to determine whether genes coding for ST-b and antibiotic resistance were transferred on the same plasmid.

MATERIALS AND METHODS

Bacterial strains. Eighteen E. coli strains that hybridized with the DNA probe encoding for ST-b were isolated from different sources in two villages in northeastern Thailand in 1982 (10). Only 1 of these 18 E. coli strains was isolated from a person or an animal with diarrhea. Isolates were serotyped by a standard method (17). Enterotoxin gene probes used in this study were derived from E. coli K-12 DH1(pCSh6) containing a multicopy recombinant plasmid encoding for ST-b (15) (provided by C. H. Lee and M. So), E. coli K-12 C600(pWD299) containing a multicopy recombinant plasmid encoding for LT (6), and E. coli K-12 C600(pR101036) containing a multicopy recombinant plasmid encoding for ST-a derived from an ETEC of porcine origin (14) (provided by S. L. Moseley). Nalidixic acid-resistant (Nal') E. coli K-12 Xac was used in bacterial conjugation experiments.

Purification of plasmid DNA. Plasmid DNA was isolated from E. coli K-12 DH1(pCCh 6) as described by So et al. (26) and cleaved with HindIII and HinfI under conditions specified by the manufacturer (Bethesda Research Laboratories, Gaithersburg, Md.). Digested DNA fragments were separated by electrophoresis on a 5% polyacrylamide gel. The 460-base-pair fragment was removed by electrophoretic transfer, ethanol precipitated, and labeled with [α-32P]deoxyribonucleotide triphosphate (New England Nuclear Corp., Boston, Mass.) by nick translation (16). The DNA probes for genes coding for LT and ST-a were constructed as previously described (20).

Plasmid DNA from ST-b E. coli was isolated by the method of Birnboim and Doly (2). Ethanol-precipitated DNA was subjected to electrophoresis in a 0.7% agarose gel in Tris-borate buffer at 100 V for 90 min. After the gel was stained with ethidium bromide and photographed under UV illumination, the DNA was transferred from the gel to nitrocellulose paper by the Southern technique (28) and examined with radiolabeled DNA probes for genes coding for LT (5), ST-a (14), and ST-b (14). Plasmid molecular weights were determined by their electrophoretic mobility relative to plasmids of known molecular weight, including pLac, pPRC122, pR1, pRP4, pR6K, and pSa (2).
method of Birnboim and Doly (2), separated by electrophoresis on a 0.7% agarose gel, transferred to nitrocellulose paper, and examined with the ST-b-coding gene probe.

## RESULTS

Plasmid DNAs of *E. coli* that hybridized with the ST-b enterotoxin probe isolated from eight people, eight pigs, a buffalo, and a water specimen were examined by Southern blot analysis (28) with DNA probes coding for LT, ST-a, and ST-b. Plasmids of between 50 and 110 megadaltons con-

### TABLE 1. ST-b *Escherichia coli* examined by Southern blot analysis with DNA probes for enterotoxins

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Source</th>
<th>Serotype</th>
<th>Characteristicsa</th>
<th>ST-II plasmid size (megadaltons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spc17</td>
<td>Adult</td>
<td>O88:H-</td>
<td>ST-b, LT', Tc'</td>
<td>62</td>
</tr>
<tr>
<td>SpD55/2B2C</td>
<td>Child</td>
<td>O88:H-</td>
<td>ST-b, Tc'</td>
<td>70</td>
</tr>
<tr>
<td>SpPC31B1A</td>
<td>Pig</td>
<td>O7:H-</td>
<td>ST-b</td>
<td>65</td>
</tr>
<tr>
<td>SpC34B1D</td>
<td>Adult</td>
<td>O?:H28</td>
<td>ST-b, Ap', Sm', Su', Tc'</td>
<td>70</td>
</tr>
<tr>
<td>SpPC19A</td>
<td>Pig</td>
<td>O7:H-</td>
<td>ST-b, Tc'</td>
<td>70</td>
</tr>
<tr>
<td>SpD11D</td>
<td>Adult</td>
<td>O9abH19</td>
<td>ST-b, ST-a'</td>
<td>50</td>
</tr>
<tr>
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<td>Pig</td>
<td>O7:H-</td>
<td>ST-b</td>
<td>110</td>
</tr>
<tr>
<td>NK18</td>
<td>Buffalo</td>
<td>O7:H4</td>
<td>ST-b, LT</td>
<td>60</td>
</tr>
<tr>
<td>NWBC40/1A1</td>
<td>Water</td>
<td>O8:H-</td>
<td>ST-b</td>
<td>100</td>
</tr>
<tr>
<td>NPD3B1B</td>
<td>Pig</td>
<td>O157:H39</td>
<td>ST-b, Tc'</td>
<td>75</td>
</tr>
<tr>
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<td>O45:H9</td>
<td>ST-b</td>
<td>100</td>
</tr>
<tr>
<td>ND3B2C</td>
<td>Adult</td>
<td>O?</td>
<td>ST-b, LT</td>
<td>75</td>
</tr>
<tr>
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<td>Pig</td>
<td>O34:H20</td>
<td>ST-b, Tc'</td>
<td>75</td>
</tr>
<tr>
<td>N26C1</td>
<td>Adult</td>
<td>O7:H4</td>
<td>ST-b, LT, Tc'</td>
<td>60</td>
</tr>
<tr>
<td>ND3B1A</td>
<td>Adult</td>
<td>O?</td>
<td>ST-b, Sm', Su', Tc'</td>
<td>62</td>
</tr>
<tr>
<td>NPD3B1A</td>
<td>Pig</td>
<td>O?</td>
<td>ST-b, Sm', Su', Tc'</td>
<td>75</td>
</tr>
<tr>
<td>NPC39B1A</td>
<td>Pig</td>
<td>O?:K2</td>
<td>ST-b</td>
<td>75</td>
</tr>
</tbody>
</table>

a Ap, Ampicillin; Cm, chloramphenicol; Su, sulfisoxazole; Tc, tetracycline.

b Hybridized with DNA probe for ST-b.

c Hybridized with DNA probe for LT.

d Hybridized with DNA probe for ST-a.

FIG. 1. Southern blot hybridization of plasmid DNA from *E. coli* that hybridized with the LT- and ST-b-coding gene probes. (a) Ethidium bromide-stained 0.7% agarose gel: lane A, SpC17; lane B, ND3B2C; lane C, CD366 that hybridized with the LT probe only; lane D, NK18; lane E, N26C1. (b) Autoradiograph of a Southern blot of the gel shown in (a) hybridized with the ST-b probe.

d FIG. 2. Southern blot hybridization of plasmid DNA from *E. coli* that hybridized with the LT- and ST-b-coding gene probes. (a) Ethidium bromide-stained 0.7% agarose gel: lane A, SpC17; lane B, ND3B2C; lane C, CD366 that hybridized with the LT probe only; lane D, NK18; lane E, N26C1. (b) Autoradiograph of a Southern blot of the gel shown in (a) hybridized with the ST-b probe.
tained genes coding for ST-b (Table 1). A Southern blot analysis of plasmid DNAs from seven ST-b E. coli isolates is shown in Fig. 1. Four ST-b E. coli isolates contained plasmids which contained genes coding for LT and ST-b (Fig. 2). One isolate from a villager contained a plasmid coding for ST-a and ST-b (Fig. 3).

Five of 11 antibiotic-resistant ST-b E. coli isolates transferred a single plasmid coding for both tetracycline resistance and ST-b. Southern blot hybridizations of the DNAs of three antibiotic-resistant ST-b E. coli isolates that transferred plasmids coding for antibiotic resistance and ST-b and two other ST-b E. coli isolates that did not are shown in Fig. 4. Two ST-b E. coli isolates containing single plasmids of 60 to 62 megadaltons that hybridized with genes coding for LT and ST-b were resistant to tetracycline. Neither isolate transferred tetracycline resistance to E. coli K-12 Xac.

FIG. 4. Southern blot hybridization of plasmid DNA isolated from antibiotic-resistant ST-b E. coli and transconjugants from their mating with E. coli K-12 Xac Nal+ (see the text). (a) Ethidium bromide-stained 0.7% agarose gel: lane A, ST-b + E. coli SpC79D1E Ap' Cm' Sm' Su' Tc'; lane B, ST-b + Tc' transconjugant of SpC79D1E and E. coli K-12 Xac Nal'; lane C, ST-b + E. coli SpPC19A Tc'; lane D, ST-b - Tc' transconjugant of SpPC19A and E. coli K-12 Xac Nal'; lane E, ST-b E. coli NPD59B1A Tc'; lane F, ST-b - Tc' transconjugant of NPD59B1A and E. coli K-12 Xac Nal'; lane G, ST-b + E. coli ND3B1A Sm' Su' Tc'; lane H, ST-b + Tc' transconjugant of ND3B1A and E. coli K-12 Xac Nal'; lane I, ST-b + E. coli NPD3B1A Sm' Su' Tc'; lane J, ST-b + Sm' Su' Tc' of NPD3B1A and E. coli K-12 Xac Nal'. (b) Autoradiograph of a Southern blot of the gel shown in (a) hybridized with the ST-b probe.

DISCUSSION

ST-b ETEC can be identified by testing E. coli in ligated piglet intestinal loops (18); however, it is not possible to perform this assay in most laboratories. It was possible, however, by using a DNA hybridization technique with a specific enterotoxin gene probe for ST-b, to study the epidemiology of bacteria containing genes coding for ST-b. This method does not necessarily identify E. coli which are capable of causing distension in pig ileal loops. In this study we identified four E. coli isolates that contained plasmids coding for both LT and ST-b. Another isolate contained a plasmid coding for both ST-a and ST-b.

Plasmids that code for ST-a (11) and ST-b are heterogeneous in size. The gene coding for ST-a is flanked immediately by inverted repeats of IS7, and this unit has the ability to transpose from one plasmid to another (27). No inverted repeat sequences in the immediate areas flanking the gene coding for ST-b have been identified (15). The heterogeneity of plasmids containing genes coding for ST-b suggests that genes coding for ST-b may also be transposable.

Plasmids coding for LT and ST-a can be cotransferred with R factors (8). Furthermore, plasmids containing genes coding for LT, ST-a, and drug resistance have been identified on the same plasmid (13). In this study, five ST-b E. coli isolates were identified which contained plasmids coding for both tetracycline resistance and ST-b, and these plasmids were transferable by bacterial conjugation to E. coli K-12. This observation suggests that the widespread use of antibiotics could increase the distribution of genes coding for ST-b as well as those coding for LT and ST-a (13).

LITERATURE CITED


