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Molecular Characterization of Strains of Enteroinvasive *Escherichia coli* O143, Including Isolates from a Large Outbreak in Houston, Texas

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A large diarrhea outbreak due to enteroinvasive *Escherichia coli* (EIEC) serogroup O143 occurring in Houston, Tex., provided the opportunity to investigate aspects of the molecular epidemiology of this and related organisms. This was done by comparing the plasmid patterns and the chromosomal restriction endonuclease digestion patterns by pulsed-field gel electrophoresis (PFGE) of EIEC from the outbreak, other *E. coli* from the same serogroup (O143), and EIEC isolated from other patients with diarrhea. Among the isolates studied, there was marked restriction fragment length polymorphism. All 3 non-O143 EIEC isolates had very different restriction endonuclease digestion patterns, as did 5 of 5 O143 non-EIEC isolates and 6 of 15 O143 EIEC isolates. Four Houston outbreak O143 EIEC isolates had the same restriction pattern as an O143 EIEC strain isolated 2 months before in Mexico and was nearly identical to another two O143 EIEC Mexican isolates. These related strains also had the same plasmid pattern; however, the presence of only a few plasmid bands, versus the 21 to 30 chromosomal bands seen with PFGE, suggests that plasmid patterns could be a less specific way to distinguish different strains. These results demonstrate that PFGE can distinguish between different *E. coli* strains of the same serogroup and phenotype. This technique can also identify relatedness within O143 EIEC, and our data suggest the spread of a strain of EIEC from Mexico to Houston, where it caused a large outbreak. PFGE may be useful to study the epidemiology of EIEC.

Enteroinvasive *Escherichia coli* (EIEC), an important cause of endemic diarrhea in South America and Eastern Europe (12), was responsible for 5% of cases of diarrhea in a study of U.S. travelers to Mexico (14) and has been implicated in occasional food-borne outbreaks of enterocolitis in adults in industrialized countries, with three outbreaks having been reported in the United States (5, 10, 13). The largest of these was an epidemic of gastroenteritis affecting at least 226 persons in 96 outbreaks throughout the country; this was subsequently shown to be due to an O124 EIEC strain and was traced to consumption of contaminated, imported French Camembert cheese (13).

We recently had the opportunity to investigate a large outbreak of diarrhea due to EIEC in Houston, Tex., that was associated with consumption of guacamole prepared by a popular local food caterer. The epidemiology of EIEC at the molecular level is not well defined in part because of the lack of a sufficiently discriminatory tool for this type of analysis. To further explore strain differences, we have compared plasmid patterns, and in addition, we used pulsed-field gel electrophoresis (PFGE), a technique recently shown to be useful in genotypic analysis of *E. coli* (1), to compare the chromosomal restriction endonuclease digestion patterns (REDPs) of EIEC from this outbreak, other *E. coli* isolates from the same serogroup (O143), and three non-O143 EIEC isolates. The aim of the study was to assess the utility of plasmid patterns and PFGE as typing methods for the differentiation of individual EIEC strains, their relationship with serotype, and their potential for use in clinical epidemiology.

MATERIALS AND METHODS

Diarrhea outbreak. An outbreak of diarrhea occurred among 370 of 1,350 persons who consumed food catered by a local restaurant in September 1985. Routine cultures of 50 stool specimens were negative for *Salmonella*, *Shigella*, and *Campylobacter* spp. EIEC was identified from cultured stool specimens from 14 of 24 patients and 1 of 12 catering employees by using a DNA probe in all (see below) and a Sereny test in the four strains described in this paper. Case control studies showed the illness to be associated with consuming guacamole.

Bacterial strains. Isolates of *E. coli* in this study were obtained from a wide geographic and temporal distribution in order to assess the discriminatory potential of the DNA techniques. Strains studied, along with their serotype, source, year of isolation, and place of origin, when known, are shown in Table 1. *E. coli* O143 isolates were kindly provided by Richard Wilson, Pennsylvania State University, University Park, and the Division of Enteric Diseases, Centers for Disease Control (CDC), Atlanta, Ga. Three O143 EIEC isolates and three O143 non-EIEC strains isolated by us in Mexico (14) were also included in the study. EIEC isolates from the Houston diarrhea outbreak were serotyped by R. Wilson. Fifteen isolates from the Houston outbreak were initially studied by plasmid pattern in 1985, and four of these were stored at −70°C (Table 1) and were available for the present study.

Plasmid pattern analysis and hybridizations. Plasmid DNA

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TABLE 1. Details of *E. coli* strains used in this study

Source and strain	Serotype	Source	Yr	DNA probe	REDP pattern
E. coli O143 from R. Wilson					
821385	O143:NM	Turkey	Unknown	Negative	B
830886	O143:NM	Unknown	Unknown	Negative	C
851792	O143:NM	Human	1985	Positive	A′
860395	O143:NM	Human	1985	Negative	D
870372	O143:H4	Chicken	Unknown	Negative	E
870653	O143:NM	Human	Unknown	Positive	A[a]
871724	O143:NM	Turkey	Unknown	Positive	F
881320	O143:H11	Bovine	Unknown	Negative	G
EIEC O143 from CDC					
3336-90 California	O143:NM	Human	Unknown	—[b]	H
80-10 Japan	O143:NM	Human	Unknown	—	I
3367-69 Kentucky	O143:NM	Human	Unknown	—	J
193-82 HSP Brazil	O143:NM	Human	Unknown	—	K
3544-89 Bulgaria	O143:NM	Human	Unknown	—	L
EIEC from travelers in Mexico (14)					
OEN 143a	O143:NM	Human	1985	Positive	A
IM 22a	O-:NM	Human	1985	Positive	M
OPB 21e	O143:NM	Human	1985	Positive	A′
OPB 85e	O-:NM	Human	1985	Positive	N
OPB 74c	O143:NM	Human	1985	Positive	A′
OEN 119a	O-:NM	Human	1985	Positive	O
EIEC from Houston outbreak					
4750	O143:NM	Human	1985	Positive	A
4674	O143:NM	Human	1985	Positive	A
4675	O143:NM	Human	1985	Positive	A
4749	O143:NM	Human	1985	Positive	A

[a] Pattern A is almost identical to pattern A′.
[b] —, Organism sent by CDC and not retested.

was isolated and analyzed by the method of Kado and Liu (6). Colony hybridizations were performed by methods previously described (4).

Chromosomal analysis by PFGE. Genomic DNA was prepared in agarose plugs (Incert Agarose; Marine Colloids Div., FMC Corp., Rockland, Maine) as previously described (7). Slices of the plugs obtained were digested with *Xba*I under conditions suggested by the manufacturer and then washed, melted, and loaded in 1.2% agarose gels (SeaPlaque GTG agarose; FMC) in Tris-borate-EDTA buffer (7). Lambda concatemers (FMC) were used as the molecular size standards. Gels were processed with a contour-clamped homogeneous electric field device (CHEF-DRII; Bio-Rad, Richmond, Calif.) by using variable pulse times over 30 h at 200 V. The REDPs were arbitrarily assigned letter designations (Table 1).

RESULTS

Plasmid analysis and hybridizations. During the diarrhea outbreak in Houston in 1985, 15 isolates with the EIEC biotype (lysine decarboxylase negative and nonmotile) were examined for total plasmid content. Total plasmid contents from five representative isolates are shown in Fig. 1. Thirteen of the 15 isolates had an identical plasmid pattern, with three plasmids of approximately 140, 50, and 6 MDa. Two of the strains had an additional plasmid, one of approximately 44 MDa and one of approximately 43 MDa. All of the 140-MDa plasmids hybridized to the EIEC DNA probe (Fig. 1), including isolates 4750, 4674, 4675, and 4749. All 15

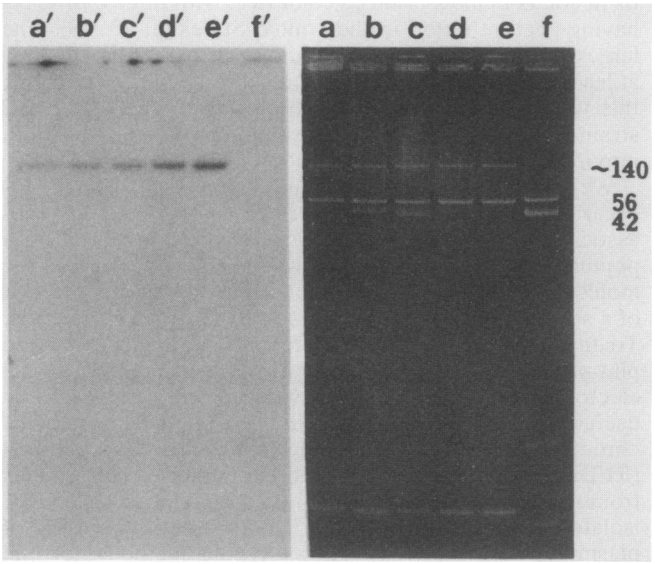

FIG. 1. On the right is an agarose gel of total plasmid DNA of five *E. coli* isolates from the Houston diarrhea outbreak (lanes a to e) and a nonenteroinvasive *E. coli* strain (lane f). On the left is an autoradiogram prepared from this gel after hybridization to the 17-kb DNA probe prepared from pMR17. Molecular masses (in megadaltons) are indicated on the right.

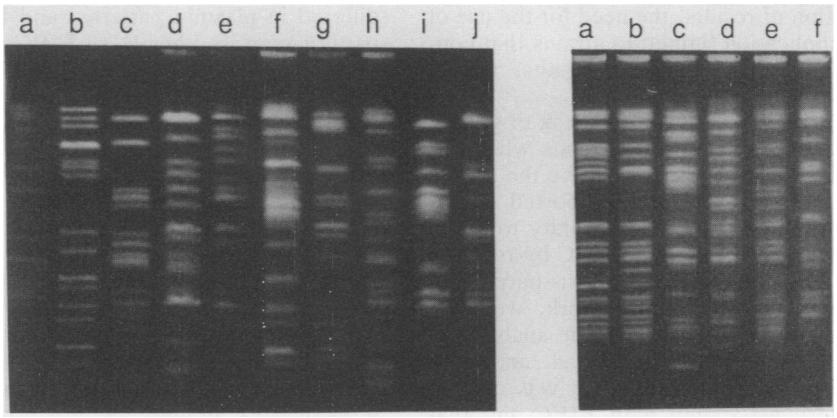

FIG. 2. PFGE of *Xba*I-digested genomic DNA of selected unrelated isolates. (Left panel) From R. Wilson, 860395 (lane a), 870372 (lane c), 830886 (lane e), 881320 (lane f), and 871724 (lane j); from Mexico, OPB 85e (lane b) and IM 22a (lane g); from CDC, 3544-89 Bulgaria (lane d), 3367-69 Kentucky (lane h), and 3369-90 California (lane i). (Right panel) From Mexico, OEN 143a (lane a); from CDC, 193-82 HSP Brazil (lane b), 3336-90 California (lane c), 3544-89 Bulgaria (lane d), 3367-69 Kentucky (lane e), and 80-10 Japan (lane f). Lane g, λ concatemers.

isolates were found to belong to serogroup O143 and were nonmotile. *E. coli* O143 strains 851792 and 870653 (from R. Wilson) and Mexican EIEC strains OEN 143a, OPB 21e, and OPB 74c (14) were also found to have the same total plasmid content (data not shown). All other *E. coli* O143 strains and the other Mexican EIEC strains had different plasmid patterns, although most of the strains had only two to three plasmids.

The four Houston outbreak isolates restudied in 1991, all six Mexican isolates, and three of eight isolates of *E. coli* O143 from R. Wilson hybridized with the EIEC probe. These last three isolates (851792, 870653, and 860395) are heretofore referred to as O143 EIEC. The strains obtained from the CDC were sent to us as EIEC and were not retested.

PFGE. Chromosomal DNA digestion of all study isolates with *Xba*I yielded between 21 and 30 discernible bands, ranging from 45 to 1,000 kb, per strain. All three non-O143 EIEC (IM 22a, OPB 85e, and OEN 119a) had REDPs distinctly different from those of all five *E. coli* O143 non-EIEC isolates (strains 821385, 830886, 860395, 870372, and 881320), the five O143 EIEC isolates sent by the CDC (strains 3336-90 California, 80-10 Japan, 3367-69 Kentucky, 193-82 HSP Brazil, and 3544-89 Bulgaria), and an O143 EIEC strain isolated from a turkey (strain 871724) (Fig. 2).

The four Houston O143 EIEC isolates from the outbreak had the same chromosomal REDP; this pattern, arbitrarily designated pattern A (Table 1), was different from that of the O143 EIEC above but was identical to that of an O143 EIEC strain (OEN 143a) isolated from a traveler to Guadalajara, Mexico (14), 2 months prior to the diarrhea outbreak (Fig. 3). These isolates differed in only one band from two other O143 EIEC strains (OPB 21e and OPB 74c) (Fig. 3) also isolated from travelers to Guadalajara 2 months before the outbreak, suggesting a clonal relationship. Isolates 851792 and 870653 also had this REDP. These isolates had been obtained as *E. coli* O143 from R. Wilson; they proved to be EIEC, and inquiry revealed that they were isolates from Guadalajara, Mexico, that had been sent to R. Wilson by one of us (J.J.M.) and were obtained from two persons whose stool isolates are included in this study (OEN 143a and OPB 21e). All of these results were reproducible, persisting after repeated electrophoresis runs. As noted above, the related strains were also shown to have the same plasmid pattern, consisting of two plasmids plus the large virulence plasmid (Fig. 1) (14).

DISCUSSION

Paramount to understanding the molecular epidemiology of a given organism is the demonstration of diversity at the subspecies level. To this end, the methods most commonly applied to *E. coli* have focused on variations in phenotypic traits, such as serotype, biochemical type, antimicrobial susceptibility, and fimbriation, and so rely on phenotypes that may not be stably expressed or have poor discriminatory value. More recently, techniques such as conventional gel electrophoresis of chromosomal restriction fragment length polymorphisms, multilocus enzyme analysis, rRNA probing, and plasmid pattern analysis have all been applied to various organisms, including *E. coli*. Unfortunately, they can be fraught with potential and real problems, including

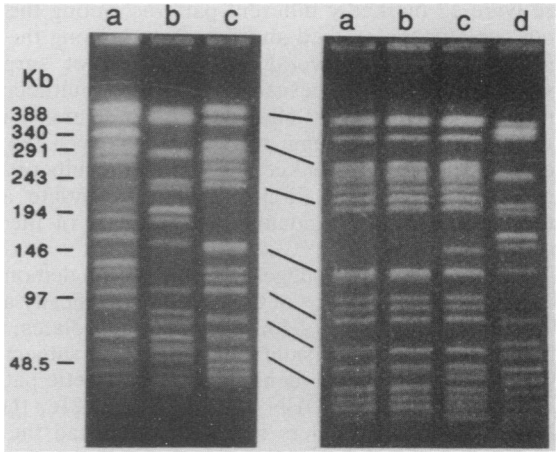

FIG. 3. PFGE of *Xba*I-digested genomic DNA of selected isolates. (Left panel) From Mexico, OPB 85 (lane a) and IM 22a (lane b); from Houston, 4750 (lane c). (Right panel) From Mexico, OEN 143a (lane b), OPB 21e (lane c), and IM 22a (lane d); from Houston, 4750 (lane a).

problematic interpretation of results, the need for the use of radioactive material, application limited to strains that contain plasmids, and difficulty reproducing results, among others (1, 12).

In 1985, we investigated a food-borne outbreak of gastroenteritis due to EIEC that affected 370 persons who consumed food provided by a caterer, proving to be the largest outbreak of EIEC in the United States reported so far (unpublished data). This gave us the opportunity to study aspects of the molecular epidemiology of EIEC by recently developed techniques now being applied for this purpose to various other organisms. In the present work, we have confirmed the usefulness of plasmid pattern analysis in studying the epidemiology of EIEC. Wanger et al., analyzing various isolates of EIEC, showed that there was no one common plasmid pattern associated with EIEC and that clonality was likely when identical plasmid patterns are observed (14). However, there was not a great deal of variation, and most isolates had only a few plasmids. Here, we have shown identical total plasmid content in 13 of 15 EIEC isolates from a common source outbreak, with two isolates apparently having acquired an additional plasmid. This pattern was identical to that of several Mexican EIEC strains previously shown to have the same pattern (14) and to that of *E. coli* strains that at the beginning of the work were thought to be unrelated but in retrospect were found to belong to the two travelers to Mexico studied here. While the use of plasmid content for molecular epidemiology of bacteria may at times be limited because of the inherent instability or nonspecific patterns of some plasmids (9), its utility was confirmed in the present study; this should encourage laboratories to consider this widely available and simple technique as the initial means of characterizing the molecular epidemiology of EIEC outbreaks.

Recently, PFGE has been used in the genomic analysis (e.g., sizing and mapping) of eucaryotic and procaryotic organisms and, in addition, to investigate the epidemiology of various organisms at the molecular level (1, 4, 7, 8, 11). In this study we have used PFGE to resolve the relatively large fragments obtained by digesting the *E. coli* chromosome with *Xba*I, a restriction endonuclease that digests it infrequently. This technique unambiguously detected genetic diversity among isolates of EIEC even when they belonged to the same serogroup (O143) and phenotype. Specifically, there were 12 markedly different patterns among the O143 *E. coli* isolates we studied and 7 patterns among the O143 EIEC isolates. This diversity perhaps is not surprising in view of previous observations utilizing multilocus enzyme electrophoresis that showed genetic diversity among *E. coli* isolates of the same serogroup and even the same serotype (2, 3), and it is in keeping with the results of Arbeit et al. (1) showing that PFGE could differentiate among epidemiologically independent *E. coli* strains of the same serotype.

Our data also strongly suggest that PFGE can demonstrate relatedness among isolates of *E. coli* and specifically among EIEC isolates. Four of the Houston outbreak isolates, which were equal by total plasmid content, were subjected to PFGE and found to have identical electrophoretic patterns. We also observed that OEN 143a and OPB 21e, the two isolates from U.S. travelers to Mexico that had the same plasmid pattern as that of the outbreak strain, had chromosomal digestion patterns identical and nearly identical, respectively, to that of the Houston outbreak strain. This pattern was different from the chromosomal digestion patterns of other Mexican EIEC strains studied here that also differed in plasmid patterns, and it was different from the pattern seen in other O143 EIEC isolates from other locations and from the pattern of O143 non-EIEC isolates. The presence of such diversity is the basis for the interpretation that the isolates with identical and almost-identical patterns represent a single strain. It does not, however, prove a direct link between the isolates found in Mexico in the summer of 1985 and the isolates causing the outbreak in Houston 2 months later. However, considering the large, mobile Mexican population residing in Houston plus the tremendous number of tourists from the United States who travel to Mexico, it is certainly conceivable that this strain was recently imported and then found its way into food in this popular Mexican restaurant in Houston. There is little known about the stability of plasmid or chromosomal patterns of EIEC in vivo, and it is possible that these identical isolates were separated epidemiologically by a longer period of time. However, the presence of variants of the plasmid pattern among the outbreak isolates plus the slight variation between the two isolates from Mexico suggest that changes can occur in a short period of time.

In summary, PFGE is a powerful tool in the genotypic profile investigation of EIEC and probably other *E. coli* strains. It reliably and reproducibly demonstrates diversity even within the same serotype and thus is capable of indicating relatedness. It should prove to be valuable in studies of the epidemiology of EIEC and other organisms.

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