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CFA/I-ST Plasmids: Comparison of Enterotoxigenic *Escherichia coli* (ETEC) of Serogroups O25, O63, O78, and O128 and Mobilization from an R Factor-Containing Epidemic ETEC Isolate

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Colonization factor antigen I (CFA/I) plays an important role in the pathogenesis of diarrhea due to enterotoxigenic *Escherichia coli*. In this study, we examined 11 CFA/I$^+$ enterotoxigenic *E. coli* from serogroups O25, O63, O78, and O128 and found that with all strains, spontaneous loss of CFA/I was associated with the loss of heat-stable toxin (ST) and with the loss of a single plasmid ranging in size from 54 to 60 megadaltons; when heat-labile toxin was lost, this was associated with the loss of another plasmid. The R factor of one strain, TX432 (O78:H12:CFA/I$^+$; ST$^+$), was found to mobilize the CFA/I-ST plasmid into *E. coli* K-12 at a frequency of 20%. These studies provide further evidence that CFA/I production is plasmid mediated in enterotoxigenic *E. coli* belonging to serogroups O25, O63, O78, and O128.

Enterotoxigenic *Escherichia coli* (ETEC) is an important cause of acute diarrhea, particularly in developing countries (1, 21, 30); ETEC has also caused sporadic outbreaks of diarrhea in more developed countries such as the United States and England (14–16, 19, 27–29). To produce diarrheal disease, these organisms must be able to attach to and proliferate on the luminal surface of the small intestine as well as produce enterotoxin(s) (6). One of the best-characterized adherence factors found on ETEC isolated from cases of human diarrhea is the fimbrial colonization factor antigen I, or CFA/I (10). Most CFA/I-producing ETEC belong to serogroups O15, O25, O63, O78, and O128 and are generally restricted to only a few serotypes within these serogroups, for example, O78:H11, O25:H42, and O63:H$^-$ (7, 20). The observation that CFA/I is plasmid mediated was first made with the prototype strain H-10407 (O78:H11:CFA/I) (11) and has more recently been shown with other O78 strains (31), an O63:H$^-$ strain (18), and with an O128ac:H12 strain (26).

The 20 strains of ETEC used in this study were originally isolated from individuals with diarrhea and have been shown in previous studies to produce CFA/I (7, 10–12, 29). To generate CFA/I-negative derivatives, these strains were serially passed on CFA agar plates (9), and individual colonies were screened for loss of stable toxin (ST) (determined by the suckling mouse assay [4]), heat-labile toxin (LT) (determined by the Y-1 adrenal cell assay [5]), and CFA/I (determined by the hemagglutination typing method described by Evans et al. [13]). CFA/I was confirmed by slide agglutination and by immunodiffusion with anti-CFA/I serum (7, 8). CFA/I-negative derivatives of CFA/I-positive ETEC strains are designated as P strains, using the suffix -P. DNA was prepared by using cleared lysates of CFA/I-positive and -negative pairs and was electrophoresed in 0.7% agarose, stained with ethidium bromide, and photographed (23). Plasmid molecular weights were determined by their electrophoretic mobility relative to plasmids of known molecular weight, including p*lac*, R1, RP4, R6K, and Sa (2).

Eleven test strains, including six different ETEC serotypes, gave rise to CFA/I-negative derivatives; all were found to have lost a single plasmid (Table 1 and Fig. 1). Six of the test strains were resistant to one or more antibiotics (as determined by disk diffusion [33]), but in no instance was antibiotic resistance lost simultaneously with the production of CFA/I. Every CFA/I-negative strain had also lost the ability to produce ST and in some cases also lost the ability to produce LT. Loss of LT production was accompanied by loss of a second plasmid. In serogroup O25, loss of CFA/I and ST production was associated with the loss of a 54-megadalton (Mdal) plasmid or a 60-Mdal plasmid (Fig.

TABLE 1. Analysis of CFA/I$^+$ and CFA/I$^-$ pairs of ETEC

Strain[a]	Source	Reference	Serotype	Antibiotic resistance[b]	CFA/I	Toxins		Mol wt of plasmid(s) lost by the P strain
						ST	LT	
PB11	Mexico	7	O25:H42	None	+	+	+	
PB11-P1				None	+	+	−	36
PB11-P2				None	−	−	−	54, 36
PB37 A8	Mexico	7	O25:H42	Ap	+	+	−	
PB37 A8-P				Ap	−	−	−	60
PB334	Mexico	7	O25:H$^-$	None	+	+	+	
PB334-P1				None	−	−	+	54
PB334-P2				None	−	−	−	54, 36
H9195	Bangladesh	7	O25:H42	None	+	+	+	
H9195-P				None	−	−	+	54
PB40	Mexico	7, 10	O63:H$^-$	Sm	+	+	+	
PB40P1				Sm	−	−	+	60
PB40P2				Sm	−	−	−	60, 50
PB200	Mexico	7, 10	O63:H$^-$	Sm	+	+	+	
PB200-P				Sm	−	−	+	60
PB312	Mexico	7	O63:H$^-$	Sm, Tc	+	+	+	
PB312				Sm, Tc	−	−	+	60
H19123	Bangladesh	7	O63:H$^-$	None	+	+	−	
H19123-P				None	−	−	−	60
H10407	Bangladesh	7, 11	O78:H11	None	+	+	+	
H10407-P				None	−	−	+	56
TX432	Texas	7, 29	O78:H12	Amp, Su, Km Tc, Sm, and Cm for both strains	+	+	−	
TX432-P					−	−	−	60
16098	Bangladesh	7	O128:H12	Tc	+	+	−	
16098-P				Tc	−	−	−	60

[a] A pair consists of a CFA/I$^+$ strain and its CFA/I$^-$ derivative; P refers to the CFA/I$^-$ derivative of an initially CFA/I$^+$ isolate of ETEC.

[b] Ap, Ampicillin; Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; Su, sulfonamide; Tc, tetracycline.

1); two of these strains gave rise to LT-negative derivatives coincident with loss of a 36-Mdal plasmid (Table 1). Strain PB-40 (O63:H$^-$) showed loss of a 50-Mdal plasmid coincident with loss of LT production. In all of the O63:H$^-$ strains, including strain H-19123 from Bangladesh, ST and CFA/I production were associated with a 60-Mdal plasmid. It can be seen from the data in Table 1 that the molecular weight of the plasmid associated with ST and CFA/I production varied, but only within a small range (54 to 60 Mdal).

E. coli strain TX432 (O78:H12:CFA/I) was resistant to multiple antimicrobial agents and produced ST but not LT; this strain was isolated during a prolonged nosocomial outbreak of diarrhea in human infants (29). A 70-Mdal plasmid encoded resistance to multiple antimicrobial agents and was transferred by broth matings (23) to *E. coli* K-12 strain J53 (*pro met* λ^+) at a frequency of 5×10^{-5} transconjugants per recipient. The ability to produce CFA/I and ST cotransferred at a frequency of 20% (one CFA/I$^+$ ST$^+$ colony in every five antibiotic-resistant colonies of strain J53) and was associated with a 60-Mdal plasmid (pTEX) (Fig. 1). To demonstrate that CFA/I production was not also dependent upon the 70-Mdal resistance plasmid, this plasmid was eliminated by using the novobiocin technique (18). Antibiotic resistance was independent of the ability to produce CFA/I.

Virulence of ETEC is determined by the pro-

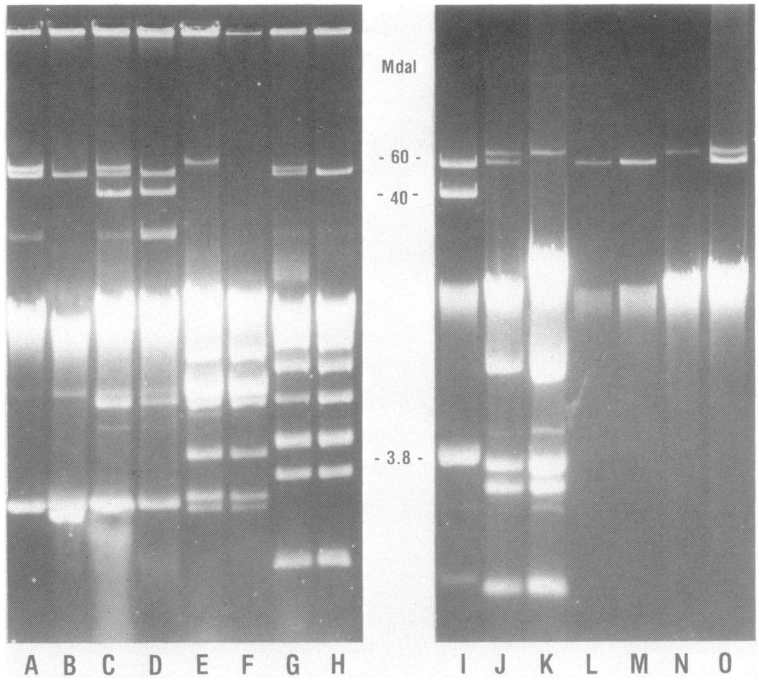

FIG. 1. Agarose gels of cleared lysates of CFA/I-producing ETEC, their corresponding CFA/I-negative P strains, and transconjugants. A, PB11-P1 (CFA/I$^+$; ST$^+$ LT$^-$); B, PB11-P2 (CFA/I$^-$; ST$^-$ LT$^-$); C, PB334 (CFA/I$^+$; ST$^+$ LT$^+$); D, PB-334-P1 (CFA/I$^-$; ST$^-$ LT$^+$); E, H9195 (CFA/I$^+$; ST$^+$ LT$^-$); F, H9195-P (CFA/I$^-$; ST$^-$ LT$^-$); G, PB37A8 (CFA/I$^+$; ST$^+$ LT$^-$); H, PB37A8-P (CFA/I$^-$; ST$^-$ LT$^-$); I, H10407; J, TX432 (CFA/I$^+$; ST$^+$ LT$^-$), multiply resistant; K, TX432-P (CFA/I$^-$; ST$^-$ LT$^-$), multiply resistant; L, transconjugant J53 (pTEX) (CFA/I$^+$; ST$^+$ LT$^-$) derived from strain shown in lane O by eliminating R factor; M, same as L; N, transconjugant which received only the R factor; O, transconjugant with R factor and pTEX; (CFA/I$^+$; ST$^+$ LT$^-$), multiply resistant.

duction of two types of virulence factors; these are the ST and LT enterotoxins and the fimbrial colonization factors (3, 6, 10, 13). The CFAs of the animal-associated ETEC exhibit specificity in that bovine-associated isolates usually produce the fimbrial adhesin K99 and the swine-associated isolates usually produce the fimbrial adhesin K88 (24, 32). Although K99-positive ETEC may be found in piglet diarrhea under special circumstances (22), K88-positive ETEC have not been isolated from bovines and neither antigenic type has been identified in cases of human diarrhea. Some human-associated ETEC serotypes produce one antigenic type of CFA (CFA/I), whereas others produce another, termed CFA/II. CFA/I and CFA/II, like K88 and K99, are plasmid-determined products of the ETEC. In most CFA/II-positive ETEC, a single plasmid encodes for CFA/II, ST, and LT (25; M. E. Peñaranda et al., manuscript in preparation; M. E. Peñaranda, D. G. Evans, B. E. Murray, and D. J. Evans, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 26th, Chicago, Ill., abstr. no. 196, 1981). However, studies on CFA/I indicate that CFA/I and ST were encoded by one plasmid and LT by another (7, 10); this has been recently confirmed by mobilization of a CFA/I-ST plasmid from an O78 ETEC with R1-19K (31) and by mobilizing a CFA/I-ST plasmid from an O128ac:H12 strain with a conjugative LT plasmid present in the clinical isolate (26). McConnell et al. (17), on the other hand, transferred a CFA/I-ST plasmid by mobilization from an O63:H$^-$ strain and found that this plasmid also coded for LT.

In this study, we have shown that in ETEC serogroups O25 and O63, as well as in serogroups O78 and O128, the production of CFA/I and ST correlates with a single plasmid which varies in molecular weight from 54 to 60 Mdal depending upon the individual isolate examined; furthermore, neither antibiotic resistance nor LT appeared to be associated with these CFA/I-ST plasmids, unlike the O63:H$^-$ ETEC reported by McConnell et al. (17). One O63 strain, H19123 from Bangladesh, was LT negative when first isolated. PB200, PB40, and PB312 all lost their CFA/I-ST plasmid without losing LT

production, and PB40-P1 (CFA/I$^-$; ST$^-$ LT$^+$) later lost LT production together with loss of a 50-Mdal plasmid.

The ability of the R factor in strain TX432 to mobilize, at high frequency, the transfer of the CFA/I-ST plasmid is of particular interest. Wachsmuth et al. (34) previously showed mobilization of a 30-Mdal ST plasmid from an isolate obtained during the same nosocomial outbreak; although they did not report on transfer of CFA/I production, they point out that most strains in the outbreak had colonization pili and they speculate on the possible significance of the presence of these pili without the 60-Mdal colonization plasmid that had been reported by Evans et al. (11). The plasmid that we examined measured 60 Mdal in size and mediated CFA/I as well as ST production; the 30-Mdal ST plasmid of Wachsmuth et al. likely represents a deletion from the larger 60-Mdal CFA/I-ST plasmid. Such deletions may partially explain why some strains produce toxin but no demonstrable CFA; deletions have been observed more frequently with CFA/II-producing strains (21st ICAAC, abstr. no. 196).

The mobilization of pTEX is also significant because, although CFA enterotoxin plasmids in clinical isolates rarely encode for antibiotic resistance, it illustrates that conjugative R factors found in nature in ETEC can mobilize virulence plasmids in vitro, an event which may also occur in vivo. This supports the concern expressed by other investigators that the widespread use of antimicrobial agents will select not only for multiply-resistant bacteria but will also select for strains that have virulence as well as resistance properties and may, furthermore, promote the dissemination of virulence plasmids even when the genes for resistance and virulence are not on the same plasmid.

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LITERATURE CITED

1. **Black, R. E., M. H. Merson, B. Rowe, P. R. Taylor, A. M. Abdul Alim, R. J. Gross, and D. A. Sack.** 1981. Enterotoxigenic *Escherichia coli* diarrhoea: acquired immunity and transmission in an endemic area. Bull. W.H.O. **59**:263–268.
2. **Bukhari, A. I., J. A. Shapiro, and S. L. Adhya (ed.).** 1977. DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Dallas, W. S., D. M. Gill, and S. Falkow.** 1979. Cistrons encoding *Escherichia coli* heat-labile toxin. J. Bacteriol. **139**:850–858.
4. **Dean, A. G., Y. C. Ching, R. G. Williams, and L. B. Harden.** 1972. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. J. Infect. Dis. **125**:407–411.
5. **Donta, S. T., H. W. Moon, and S. C. Whipp.** 1974. Detection of heat-labile *E. coli* enterotoxin with the use of adrenal cells in tissue culture. Science **183**:334–336.
6. **Elwell, L. P., and P. L. Shipley.** 1980. Plasmid-mediated factors associated with virulence of bacteria to animals. Annu. Rev. Microbiol. **34**:465–496.
7. **Evans, D. G., and D. J. Evans, Jr.** 1978. New surface-associated heat-labile colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serotypes O6 and O8. Infect. Immun. **21**:638–647.
8. **Evans, D. G., D. J. Evans, Jr., S. Clegg, and J. A. Pauley.** 1979. Purification and characterization of the CFA/I antigen of enterotoxigenic *Escherichia coli*. Infect. Immun. **25**:738–748.
9. **Evans, D. G., D. J. Evans, Jr., and W. Tjoa.** 1977. Hemagglutination of human group A erythrocytes by enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. Infect. Immun. **18**:330–337.
10. **Evans, D. G., D. J. Evans, Jr., W. S. Tjoa, and H. L. DuPont.** 1975. Detection and characterization of colonization factor of enterotoxigenic *Escherichia coli* isolated from adults with diarrhea. Infect. Immun. **19**:727–736.
11. **Evans, D. G., R. P. Silver, D. J. Evans, Jr., D. G. Chase, and S. L. Gorbach.** 1975. Plasmid-controlled colonization factor associated with virulence in *Escherichia coli* enterotoxigenic for humans. Infect. Immun. **12**:656–667.
12. **Evans, D. J., Jr., D. G. Evans, H. L. DuPont, F. Orskov, and I. Orskov.** 1977. Patterns of loss of enterotoxigenicity by *Escherichia coli* isolated from adults with diarrhea: suggestive evidence for an interrelationship with serotype. Infect. Immun. **17**:105–111.
13. **Evans, D. J., Jr., D. G. Evans, L. S. Young, and J. Pitt.** 1980. Hemagglutination typing of *Escherichia coli*: definition of seven hemagglutination types. J. Clin. Microbiol. **12**:235–242.
14. **Gorbach, S. L., B. H. Kean, D. G. Evans, D. J. Evans, and D. Bessudo.** Travelers' diarrhea and toxigenic *Escherichia coli*. N. Engl. J. Med. **292**:933–936.
15. **Lee, J. A., and B. H. Kean.** 1978. International conference on the diarrhea of travelers—new directions in research: a summary. J. Infect. Dis. **137**:355–369.
16. **Luke, R. K. J., N. J. Ryan, G. L. Barnes, R. F. Bishop, and R. D. B. Dayaratyne.** 1978. Enterotoxin-producing *E. coli* and diarrhea among newborn babies in Darwin. Med. J. Aust. **1**:170–171.
17. **McConnell, M. M., H. R. Smith, G. A. Willshaw, A. M. Field, and B. Rowe.** 1981. Plasmids coding for colonization factor I and heat-stable enterotoxin production isolated from enterotoxigenic *Escherichia coli*: comparison of their properties. Infect. Immun. **32**:927–936.
18. **McHugh, G. L., and M. N. Swartz.** 1977. Elimination of plasmids from several bacterial species by novobiocin. Antimicrob. Agents Chemother. **12**:423–426.
19. **Merson, M. H., G. K. Morris, D. A. Sack, J. G. Wells, J. C. Feeley, R. B. Sack, W. B. Creech, A. Z. Kapikian, and E. J. Gangarosa.** 1976. Travelers' diarrhea in Mexico: a prospective study of physicians and family members attending a conference. N. Engl. J. Med. **294**:1299–1305.
20. **Merson, M. H., F. Orskov, I. Orskov, R. B. Sack, I. Huq, and F. Koster.** 1979. Relationship between enterotoxin production and serotype in enterotoxigenic *E. coli*. Infect. Immun. **23**:325–329.
21. **Merson, M. H., R. B. Sack, S. Islam, G. Saklayen, N. Huda, I. Huq, A. W. Zulich, R. H. Yolken, and A. Z. Kapikian.** 1980. Disease due to enterotoxigenic *E. coli* in Bangladeshi adults: clinical aspects and a controlled trial of tetracycline. J. Infect. Dis. **141**:702–711.
22. **Moon, H. W., B. Nagy, R. E. Isaacson, and I. Orskov.** 1977. Occurrence of K99 antigen on *Escherichia coli* isolated from pigs and colonization of pig ileum by K99 enterotoxigenic *E. coli* from calves and pigs. Infect. Immun. **15**:614–620.
23. **Murray, B. E., and R. C. Moellering.** 1979. Aminoglycoside-modifying enzyme among clinical isolates of *Acinetobacter calcoaceticus* subsp. *antitratus* (*Herellea vagini-*

cola); Explanation for high level resistance. Antimicrob. Agents Chemother. **15**:190–199.

24. **Orskov, I., F. Orskov, H. W. Smith, and W. J. Sojka.** 1975. The establishment of K99, a thermolabile transmissible *Escherichia coli* antigen, previously called "Kco" possessed by calf and lamb enteropathogenic strains. Acta Pathol. Microbiol. Scand. Sect. B **83**:31–36.
25. **Peñaranda, M. E., M. B. Mann, D. G. Evans, and D. J. Evans.** 1980. Transfer of an ST:LT:CFA/II plasmid into *E. coli* K12 strain RR1 by cotransformation with pSC301 plasmid DNA. FEMS Microbiol. Lett. **8**:251–254.
26. **Reis, M. H. L., M. H. T. Afonso, L. R. Trabulsi, A. J. Mazaitis, R. Maas, and W. K. Maas.** 1980. Transfer of a CFA/I-ST plasmid promoted by a conjugative plasmid in a strain of *Escherichia coli* of serotype O128ac:H12. Infect. Immun. **29**:140–143.
27. **Rosenberg, M. L., J. P. Koplan, I. K. Wachsmuth, J. G. Wells, E. J. Gangarosa, R. L. Guerrant, and D. A. Sack.** 1977. Epidemic diarrhea at Crater Lake from enterotoxigenic *E. coli*. A large waterborne outbreak. Ann. Intern. Med. **86**:714–718.
28. **Rowe, B., R. J. Gross, S. M. Scotland, A. E. Wright, G. W. Shillon, and N. J. Hunter.** 1978. Outbreak of infantile enteritis caused by enterotoxigenic *Escherichia coli* O6:H16. J. Clin. Pathol. **31**:217–219.
29. **Ryder, R. W., I. K. Wachsmuth, A. E. Baxton, D. G. Evans, H. L. DuPont, E. Mason, and F. F. Barrett.** 1976. Infantile diarrhea produced by a heat stable enterotoxigenic *E. coli*. N. Engl. J. Med. **295**:849–853.
30. **Sack, D. A., D. C. Kaminsky, R. B. Sack, I. A. Wamola, F. Orskov, I. Orskov, R. C. B. Slack, R. R. Arthur, and A. Z. Kapikian.** 1977. Enterotoxigenic *Escherichia coli* diarrhea of travelers: a prospective study of American Peace Corps volunteers. Johns Hopkins Med. J. **141**:63–70.
31. **Smith, H. R., A. Cravioto, G. A. Willshaw, M M. McConnell, S. M. Scotland, R. J. Gross, and B. Rowe.** 1979. A plasmid coding for the production of colonization factor antigen I and heat stable enterotoxin in strains of *Escherichia coli* of serotype O78. FEMS Microbiol. Lett. **6**:255–260.
32. **Smith, H. W., and M. Linggood.** 1971. Observations on the pathogenic properties of the K88, Hly, and Ent plasmids of *E. coli* with special reference to porcine diarrhea. J. Med. Microbiol. **4**:467–486.
33. **Steers, E., E. F. Foltz, and B. J. Graves.** 1959. An inocular replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. **9**:307–311.
34. **Wachsmuth, I. K., S. Falkow, and R. W. Ryder.** 1976. Plasmid-mediated properties of a heat-stable enterotoxin producing *Escherichia coli* associated with infantile diarrhea. Infect. Immun. **14**:403–407.