INFECTION AND IMMUNITY, Jan. 1985, p. 338–340
0019-9567/85/010338-03$02.00/0

Vol. 47, No. 1

Enterotoxin Production, Presence of Colonization Factor Antigen I, and Adherence to HeLa Cells by *Escherichia coli* O128 Strains Belonging to Different O Subgroups

BEATRIZ E. C. GUTH,* M. LOURDES M. SILVA, ISABEL C. A. SCALETSKY, M. REGINA F. TOLEDO, AND LUIZ R. TRABULSI

Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, CP 20342, São Paulo, SP, Brazil

Received 3 July 1984/Accepted 28 September 1984

Strains of three subgroups of *Escherichia coli* O128 were studied. Enterotoxin production was observed in 30 (91%) O128ac strains, whereas strains of subgroups O128ab and O128ad were not toxigenic. CFA/I was only found in two serotypes of subgroup O128ac, all of them producing heat-stable enterotoxin except for one which produced both toxins. None of the strains studied produced CFA/II. In a binding test with HeLa cells, localized adherence was found only in strains of subgroup O128ab; diffuse adherence occurred in strains of subgroup O128ac. As flagellar antigens were specific in subgroups ab and ac and toxin production was observed only in subgroup ac, the present results suggest that subgroup and serotype are useful markers for O128 strains that are enterotoxigenic or enteropathogenic.

Escherichia coli strains of serogroup O128 were first isolated by Taylor and Charter from cases of infantile diarrhea (25). These authors described six serotypes (O:H types) in this serogroup: H^- (nonmotile), H2 (most common), H8, H9, H10, and H12. The H10 and H12 serotypes had O antigens that were slightly different from the others and failed to ferment sucrose and raffinose. Taylor and Charter's strains, as well as other strains, were studied by Ewing et al. (10), who verified that serogroup O128 could be divided into at least three subgroups designated 128a,128b; 128a,128c, and 128a,128d. In each of these subgroups would occur different O:H types; the ones most frequently isolated from clinical cases were O128ab:H2, O128ab:H7, O128ab:H8, and O128ac:H12. *E. coli* strains of serogroup O128ad were all isolated from kittens (10). *E. coli* strains of serogroup O128 are referred to in some papers as enterotoxigenic *E. coli*, in others as classical enteropathogenic *E. coli* (EPEC), and for Ørskov et al. they may be enterotoxigenic *E. coli* or EPEC (16). However, it was observed in our laboratory that the O128 strains producing enterotoxin (ST) were all O128ac (17). The aims of this paper are to report the results of studies carried out with strains of the three subgroups of E. coli O128 in regard to production of ST and heat-labile enterotoxin (LT), the presence of colonization factor antigens, ability to adhere to HeLa cells, and carbohydrate fermentation patterns.

Fifty-six strains of *E. coli* of serogroup O128 were studied. Seventeen strains belonged to subgroup O128ab, 33 were O128ac, and 6 were O128ad. The serotypes of the strains are shown in Table 1. Thirty strains (6 O128ab and 24 O128ac) were isolated in our laboratory between 1977 and 1982 from feces of children with diarrhea, except for one which was isolated from sewage. Four strains of O128ac were from Bangladesh, and 22 strains (11 O128ab, 5 O128ac, and 6 O128ad) were from the Centers for Disease Control, Atlanta, Ga. All strains were characterized for O and H antigens as described by Edwards and Ewing (7), using antisera to *E. coli* O groups 1 to 164 and flagellar antigens H1 to H49. The O subgroups were determined by using absorbed antisera. Fermentation patterns of the strains were determined as recommended by Edwards and Ewing (7). The sugars used were lactose, sucrose, mannitol, dulcitol, salicin, adonitol, sorbitol, arabinose, raffinose, rhamnose, maltose, xylose, and esculin.

All isolates were tested for production of LT by the Y1-adrenal cell culture assay (6), in miniculture plates, and for production of ST by the infant mouse assay (4). Strains were cultivated by aeration in Casamino Acids-yeastextract medium (9) for the toxin assay. The colonization factor antigens I and II (CFA/I and CFA/II) were detected by mannose-resistant hemagglutination of human and bovine erythrocytes (8). Their presence was confirmed by slide agglutination tests with specific CFA/I and CFA/II antisera. The adherence patterns to HeLa cells were studied as described by Scaletsky et al. (22).

Enterotoxin production was observed in 30 strains of subgroup O128ac (91%): 28 produced only ST, and 2 strains of serotype O128ac:H12 produced LT and ST (LTST). Three strains belonging to serotypes O128ac:H^-, O128ac:H12, and O128ac:H25 were not toxigenic; neither were all the strains belonging to subgroups O128ab and O128ad.

Strains of subgroup O128ac producing only ST were previously described in our laboratory (17–19). Other authors referred to the production of ST (2, 5, 14, 20), LT (2), or LT and ST (3, 13) by *E. coli* O128 without mentioning the subgroup of the strains. It is interesting to observe that all O128ac strains that we have isolated in Brazil produce only ST, whereas the two strains producing LTST were from Bangladesh. The proportion of strains of *E. coli* O128ac producing ST is high among the isolates from cases of infantile diarrhea in São Paulo. This subgroup has contributed 50% of the ST-producing strains isolated during the period 1980 to 1982 (B. E. C. Guth, unpublished data).

CFA/I was found in 14 (42%) of 33 O128ac strains. The O128ab and O128ad strains were negative for CFA/I, and none of the strains studied produced CFA/II. CFA/I was only found in serotypes O128ac:H7 (2 of 5 strains) and

* Corresponding author.

TABLE 1. Subgroups and serotypes of *E. coli* O128 strains

Subgroup	Serotype	No. of strains tested
O128ab	O128ab:H1	2
	O128ab:H2	7
	O128ab:H6	1
	O128ab:H8	1
	O128ab:H35	6
O128ac	O128ac:H$^-$	3
	O128ac:H7	5
	O128ac:H12	14
	O128ac:H21	5
	O128ac:H25	1
	O128ac:H27	5
O128ad	O128ad:H$^-$	1
	O128ad:H2	2
	O128ad:H12	2
	O128ad:H47	1

O128ac:H12, in which it was most frequently found (12 of 14 strains). All strains showing CFA/I produced only ST, except for one which produced LT and ST. The simultaneous production of CFA/I and ST was previously described in some serotypes of O128 strains by Scotland et al. (23) and Reis et al. (18).

The present results and data from others suggest that there is a relationship between the presence of CFA/I and ST and the serotype of the O128 strains. Scotland et al. (23) described CFA/I and ST in serotypes O128:H7, O128:H10, and O128:H12; Reis et al. (18) found them in serotype O128ac:H12; and we found CFA/I and ST in serotypes O128ac:H7 and O128ac:H12. Other O128 serotypes have not been related with the simultaneous expression of CFA/I and ST. Serotypes O128:H18, O128:H20, O128:H21, and O128:H27 were described as ST producers without forming CFA/I (18, 23). Similar results were found in this work for strains of serotypes O128ac:H$^-$, O128ac:H21, and O128ac:H27.

One strain of serotype O128ac:H12 producing LT and ST had CFA/I. To date, the association of LTST and CFA/I has only been described in other serogroups of *E. coli* (2, 13, 21). Different colonization factors, CFA/II and CFA/III, were found in O128 strains producing both toxins (3, 13).

The localized adherence pattern was only found in three strains of subgroup O128ab: serotypes O128ab:H1 (two strains) and O128ab:H2 (one strain). The diffuse adherence pattern was found in six enterotoxigenic strains of subgroup O128ac: serotypes O128ac:H$^-$ (one strain), O128ac:H12 (one strain), and O128ac:H21 (four strains). All of these strains did not produce CFA/I except for one of serotype O128ac:H12.

TABLE 2. Enterotoxin production, presence of colonization factors, and adherence to HeLa cells in subgroups of *E. coli* O128

Subgroup	Characteristics shown by strains of *E. coli* (%)						
	LT	ST	LTST	CFA/I	CFA/II	LA[a]	DA[b]
O128ab	−	−	−	−	−	+(18)	−
O128ac	−	+(85)	+(6)	+(42)	−	−	+(18)
O128ad	−	−	−	−	−	−	−

[a] LA, Localized adherence pattern.
[b] DA, Diffuse adherence pattern.

TABLE 3. Fermentation patterns found in *E. coli* serotypes O128ab:H2, O128ac:H$^-$, and O128ac:H12 and relationship with enterotoxin production or localized adherence (LA) pattern

Serotype	No. of strains tested	Toxin production or LA pattern (no.)	Fermentation pattern[a] (no.)						
			Ram	Sor	Suc	Dul	Raf	Sal	Esc
O128ab:H2	7	NA[b] (6)	+	+(4)	+	+(4)	+	+	+
		LA (1)	+	−	+	+	+	−	−
O128ac:H$^-$	3	ST (2)	+	+	+	+	+	+	+
		NT[c] (1)	+	+	−	+	−	−	−
O128ac:H12	14	ST (11)	+	+	−	+	−	−	−(10)
		LTST (2)	+	+	+	+	+	+	+
		NT (1)	−	+	−	+	−	−	−

[a] +, Positive reaction within 48 h; −, negative reaction in 8 days. Abbreviations: Ram, rhamnose; Sor, sorbitol; Suc, sucrose; Dul, dulcitol; Raf, raffinose; Sal, salicin; Esc, esculin.
[b] NA, No adherence.
[c] NT, Non-toxigenic.

Localized adherence was described as characteristic of EPEC serogroups O55, O86, O111ab, O119, O125, O128ab, and O142 (22). In each of these serogroups, only specific serotypes related to cases of infantile diarrhea showed this adherence pattern (I. C. A. Scaletsky, M. L. M. Silva, M. R. F. Toledo, and L. R. Trabulsi, unpublished data). Diffuse adherence was also previously found in strains isolated from urine (22), and more recently in strains of some serotypes of EPEC serogroups (I. C. A. Scaletsky, M. L. M. Silva, M. R. F. Toledo, and L. R. Trabulsi). Therefore, this pattern seems not to be related to a specific class of *E. coli*.

A summary of the characteristics mentioned above, which were observed in each subgroup of *E. coli* O128, is shown in Table 2.

Several patterns were found for carbohydrate fermentation in each O128 subgroup. All strains studied fermented lactose, arabinose, maltose, mannitol, and xylose. None of them fermented adonitol. Although each serotype had a quite homogeneous fermentation pattern, some interesting findings should be stressed. One serotype of subgroup O128ab and two serotypes of subgroup O128ac had fermentation patterns related to localized adherence and toxin production, respectively (Table 3). Although a relationship among these characteristics seems to exist in specific serotypes, no definitive conclusions can be drawn in regard to fermentation properties and pathogenicity.

Although certain flagellar antigens such as H7, H8, and H12 can be found in strains belonging to different O128 subgroups (10), we verified that subgroups ab and ac have specific serotypes.

Studies with O128 enterotoxigenic strains isolated in different countries showed that most of them have the same flagellar antigens we found (5, 15, 21, 23). Other flagellar antigens, H10, H18, H20, and H49, were also reported (2, 23).

Enteropathogenic O128 strains isolated in Brazil have flagellar antigens H2 and H35. These serotypes have been described by other authors (1, 12, 24). Moreover, antigens H1, H8, H10, H12, H33, and H45 were also related with nonenterotoxigenic O128 strains (11, 24).

The results obtained in the present work suggest that in serogroup O128 there is a strong relationship between subgroup, serotype, and the enterotoxigenic or enteropathogenic character of *E. coli* strains. Therefore, in subgroup O128ac, enterotoxigenic *E. coli* strains are found in specific serotypes or bioserotypes. If localized adherence is a characteristic of EPEC, then strains belonging to specific sero-

types or bioserotypes of subgroup O128ab that present localized adherence patterns should be considered EPEC. At present, no conclusions can be drawn in regard to O128ad strains.

This work was supported by IV Plano Integrado de Genética grant 402530/82 from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Financiadora de Estudos e Projetos and Fundação de Amparo à Pesquisa do Estado de São Paulo.

LITERATURE CITED

1. **Cravioto, A., R. J. Gross, S. M. Scotland, and B. Rowe.** 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol. **3**:95–99.
2. **Cravioto, A., S. M. Scotland, and B. Rowe.** 1982. Hemagglutination activity and colonization factor antigens I and II in enterotoxigenic and non-enterotoxigenic strains of *Escherichia coli* isolated from humans. Infect. Immun. **36**:189–197.
3. **Darfeuille, A., B. Lafeuille, B. Joly, and R. Cluzel.** 1983. A new colonization factor antigen (CFA/III) produced by enteropathogenic *Escherichia coli* O128:B12. Ann. Microbiol. (Inst. Pasteur) **134A**:53–64.
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. **De Boy, J. M. II, I. K. Wachsmuth, and B. R. Davis.** 1980. Serotypes of enterotoxigenic *Escherichia coli* isolated in the United States. Infect. Immun. **29**:361–368.
6. **Donta, S. T., H. W. Moon, and S. C. Whipp.** 1974. Detection of heat-labile *Escherichia coli* enterotoxin with the use of adrenal cells in tissue culture. Science **183**:334–336.
7. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of the *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
8. **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 serogroups O6 and O8. Infect. Immun. **21**:638–647.
9. **Evans, D. G., D. J. Evans, Jr., and N. F. Pierce.** 1973. Differences in the response of rabbit small intestine to heat-labile and heat-stable enterotoxins of *Escherichia coli*. Infect. Immun. **7**:873–880.
10. **Ewing, W. H., B. R. Davis, and T. S. Montague.** 1963. Studies on the occurrence of *Escherichia coli* serotypes associated with diarrheal disease. Department of Health, Education, and Welfare publication no. (CDC) 75-8287, Centers for Disease Control, Atlanta, Ga.
11. **Gurwith, M., D. Hinde, R. Gross, and B. Rowe.** 1978. A prospective study of enteropathogenic *Escherichia coli* in endemic diarrheal disease. J. Infect. Dis. **137**:292–297.
12. **Levine, M. N., E. H. Bergquist, D. R. Nalin, D. H. Waterman, R. B. Hornick, C. R. Young, and S. Sotman.** 1978. *Escherichia coli* strains that cause diarrhoea but do not produce heat-labile or heat-stable enterotoxins and are non-invasive. Lancet **i**:1119–1122.
13. **Levine, M. M., P. Ristaino, R. B. Sack, J. B. Kaper, F. Ørskov, and I. Ørskov.** 1983. Colonization factor antigens I and II and type 1 somatic pili in enterotoxigenic *Escherichia coli*: relation to enterotoxin type. Infect. Immun. **39**:889–897.
14. **Merson, M. H., B. Rowe, R. E. Black, I. Huq, R. J. Gross, and A. Eusof.** 1980. Use of antisera for identification of enterotoxigenic *Escherichia coli*. Lancet **ii**:222–224.
15. **Ørskov, F., I. Ørskov, D. J. Evans, Jr., R. B. Sack, D. A. Sack, and T. Wadström.** 1976. Special *Escherichia coli* serotypes among enterotoxigenic strains from diarrhoea in adults and children. Med. Microbiol. Immunol. **162**:73–80.
16. **Ørskov, I., F. Ørskov, B. Jann, and K. Jann.** 1977. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. Bacteriol. Rev. **41**:667–710.
17. **Reis, M. H. L., A. F. P. Castro, M. R. F. Toledo, and L. R. Trabulsi.** 1979. Production of heat-stable enterotoxin by the O128 serogroup of *Escherichia coli*. Infect. Immun. **24**:289–290.
18. **Reis, M. H. L., B. E. C. Guth, T. A. T. Gomes, J. Murahovschi, and L. R. Trabulsi.** 1982. Frequency of *Escherichia coli* strains producing heat-labile toxin or heat-stable toxin or both in children with and without diarrhea in São Paulo. J. Clin. Microbiol. **15**:1062–1064.
19. **Reis, M. H. L., D. P. Matos, A. F. Pestana de Castro, M. R. F. Toledo, and L. R. Trabulsi.** 1980. Relationship among enterotoxigenic phenotypes, serotypes, and sources of strains in enterotoxigenic *Escherichia coli*. Infect. Immun. **28**:24–27.
20. **Sack, D. A., M. H. Merson, J. G. Wells, R. B. Sack, and G. K. Morris.** 1975. Diarrhea associated with heat-stable enterotoxin-producing strains of *Escherichia coli*. Lancet **ii**:239–241.
21. **Sack, R. B.** 1975. Human diarrheal disease caused by enterotoxigenic *Escherichia coli*. Annu. Rev. Microbiol. **29**:333–353.
22. **Scaletsky, I. C. A., M. L. M. Silva, and L. R. Trabulsi.** 1984. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. Infect. Immun. **45**:534–536.
23. **Scotland, S. M., N. P. Day, A. Cravioto, L. V. Thomas, and B. Rowe.** 1981. Production of heat-labile or heat-stable enterotoxins by strains of *Escherichia coli* belonging to serogroups O44, O114, and O128. Infect. Immun. **31**:500–503.
24. **Scotland, S. M., N. P. Day, and B. Rowe.** 1980. Production of a cytotoxin affecting Vero cells by strains of *Escherichia coli* belonging to traditional enteropathogenic serogroups. FEMS Microbiol. Lett. **7**:15–17.
25. **Taylor, J., and R. E. Charter.** 1955. *Escherichia coli* O128 causing gastroenteritis of infants. J. Clin. Pathol. **8**:276–281.