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Relationship Between Enterotoxin Production and Serotype in Enterotoxigenic *Escherichia coli*

MICHAEL H. MERSON,† FRITS ØRSKOV,[1] IDA ØRSKOV,[1] R. BRADLEY SACK,[2]* IMDADUL HUQ,[3] AND FREDERICK T. KOSTER[2]

Department of Medicine, Johns Hopkins University and Baltimore City Hospitals, Baltimore, Maryland 21218;[2] Collaborative Center for Reference and Research on Escherichia, World Health Organization, Statens Seruminstitut, Copenhagen, Denmark;[1] and Cholera Research Laboratory, Dacca, Bangladesh[3]

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We examined the relationship between serotype and enterotoxin production in 109 enterotoxigenic *Escherichia coli* strains isolated from 109 patients with severe cholera-like diarrhea in Dacca, Bangladesh. Of 69 strains producing both heat-labile and heat-stable toxins, 59 (86%) belonged to the one of four O serogroups, and 56 (81%) of these strains belonged to one of six O:K:H serotypes. In contrast, 34 strains producing only heat-stable toxin were distributed among 15 O serogroups, and six strains producing only heat-labile toxin were distributed among six O serogroups. Twelve strains producing heat-labile and heat-stable toxins and five strains producing heat-stable toxin were found which had the same serotype (O78:K$^-$:H12) and biotype. It appears that at least in one geographic setting *E. coli* strains producing both heat-labile and heat-stable toxins are more restricted in their O groups and O:K:H serotypes than *E. coli* that produce only heat-stable toxin and that certain serobiotypes may commonly include strains which produce both toxin types.

Enterotoxigenic *Escherichia coli* (ETEC) are now a well-recognized worldwide cause of acute watery diarrhea. Previous studies have shown that in Bangladesh these organisms frequently cause an illness indistinguishable from cholera (7, 14, 17). The property of enterotoxin production is known to be plasmid mediated (4, 21); enterotoxigenic strains may produce either a heat-labile toxin (LT), a heat-stable toxin (ST), or both LT and ST (LT-ST).

Accumulating evidence suggests that ETEC may commonly belong to a small number of serogroups and serotypes (10–12). This observation is potentially important since presently available tests for detection of LT- and ST-producing strains (15) are not applicable to most laboratories and the development of a serological capability for identification would greatly facilitate diagnosis of ETEC-mediated disease. We report here the serotype and enterotoxin properties of 109 ETEC strains isolated from 109 patients admitted to our hospital. Our observations suggest that this restriction of ETEC strains to select serogroups and serotypes applies particularly to those producing LT and ST but that there may be a few serotypes that are common to both toxin types.

† Present address: Bacterial and Venereal Infections, World Health Organization, Geneva, Switzerland.

MATERIALS AND METHODS

Admission stool specimens from 176 male adults with acute, cholera-like diarrhea who were admitted to the Cholera Research Hospital, Dacca, Bangladesh, in October through December 1976 were plated on MacConkey agar. After overnight incubation at 37°C 10 lactose-positive organisms with typical *E. coli* morphology were selected from each plate and streaked onto nutrient agar slants for storage. Within 1 month after isolation each isolate was grown in Trypticase soy broth with 0.6% yeast extract in an 18- to 24-h shake culture at 37°C. Broth cultures were centrifuged at 6,000 rpm, and supernatants were tested for LT by the Chinese hamster ovary assay (3) and for ST by the infant mouse assay (6).

Enterotoxin-producing organisms were isolated from 109 patients. In 100 (92%) patients, 9 or 10 of the 10 isolates were enterotoxigenic. From each of these patients one enterotoxigenic isolate was identified as *E. coli* (1). O and H antigens were then determined by routine procedures (9), and K determination was carried out by countercurrent immunoelectrophoresis as recently described (8, 20). Biochemical examinations for biotype determination were done by standard procedures (5).

RESULTS

A total of 69 strains produced both LT and ST, 34 produced only ST, and 6 produced only LT. The relationship between O serogroups and enterotoxin types of the 109 strains is shown in

Table 1. The 69 LT-ST strains were distributed among ten O groups, and 59 (86%) of the 69 strains belonged to one of four O groups (O6, O8, O78, O115), which will subsequently be referred to as major LT-ST O groups. In contrast, the 34 ST strains were distributed among 15 O groups, and a significantly lower number of them (eight [24%]) were included in the four major LT-ST O groups ($\chi^2 = 35.7$; $P < 0.001$). The two most commonly isolated serogroups of the ST strains were O78 and O128 (five in each). The six LT strains had different O groups, and one of these six had a major LT-ST O group.

Table 2 presents the O:K:H serotypes by enterotoxin type. All 9 LT-ST O6 strains were O6:K15:H16 with the same biopattern, and all 18 LT-ST O8 strains were O8:K40:H9 of one biotype. Of 25 O78 strains, 6 belonged to bioserotype O78:K$^-$:(H11), and 17 belonged to bioserotype O78:K$^-$:H12; five of this last type were the only ST strains among O78 strains. Of 11 O115 LT-ST strains, 5 belonged to bioserotype O115:K$^-$:H40, and 6 belonged to bioserotype O115:K$^-$:(H51); two ST O115 strains belonged to one bioserotype, O115:K$^-$:H21. All O128 strains were ST only, three of them belonging to the same bioserotype (O128:K$^-$:H21) which did not have the serological characteristics of the classical enteropathogenic O128 serogroup (O128:K$^-$:H2).

Table 3 shows the biotypes of eight of the O:K:H serotypes mentioned above and two strains of O25:K7:H42. For the first five of those listed the pattern has been reported previously (11), and in this table the results of the ornithine tests are also included. It is evident that in the present material the same biotype was often found within a certain O:K:H serotype.

There was no temporal or geographic clustering of any of the *E. coli* serogroups or serotypes.

TABLE 1. *O group and enterotoxin type of E. coli strains isolated from 109 cases of diarrhea at Dacca, Bangladesh, in 1976*

O group	No. with the following enterotoxin type:		
	LT-ST	ST only	LT only
O4		1	
O6	9		
O7		1	
O8	19[a]	1	1
O15	1		
O20	1	3[b]	
O25	2		
O29		2	
O34		2	
O48			1
O63	2	2	
O78	20	5	
O85	2	1	
O96			1
O114		1	1
O115	11	2	
O123		1	
O126	1	1	
O128		5	
O148		1	
O159			1
OX2			1
Negative O1-O163	1	3	
Spontaneous agglutination		2	

[a] Includes one O8,O60 that was LT-ST.
[b] Includes three O20,O153 that were ST only.

DISCUSSION

In a previous review of serotypes of ETEC isolated from different countries, Ørskov et al. (10) found that the most frequently identified O groups of these strains were O6, O8, O15, O25, O78, O115, and O128 and that five O:H combinations were found with particularly high frequency (O6:H16, O8:H9, O15:H11, O78:H11, and O78:H12). They subsequently reported that the O6:H16 and most of the O8:H9 strains had the same K antigen (K15 and K40, respectively) and that enterotoxigenic strains of nine serotypes, including the five mentioned above, had a predictable biotype (11). Rowe et al. (13) also reported their observation of a limited number of serogroups for ETEC strains, which included some of the same serogroups cited by Ørskov et al. Scotland et al. (19) reported four different biotypes among 20 ETEC O6:H16 strains isolated from humans; three of these fitted the biotype given in an earlier paper by Ørskov and Ørskov (11) and the fourth was represented by only one strain, which had a K15 antigen similar to the others. None of these studies, however, examined the relationship between serogroups or serotypes and type of enterotoxin production.

Our results represent the largest number of serotyped ETEC reported from a single geographic location to date. We have confirmed the initial observations of Ørskov et al. and Rowe et al. and have also shown that the close relationship between serogroup and enterotoxin type is found primarily in LT-ST ETEC strains. A total of 90% of LT-ST ETEC strains isolated from our hospitalized patients were in the seven O groups originally cited by Ørskov et al. and 86% were in four of the seven (O6, O8, O78, O115). In contrast, the 34 ST strains were included in 15 O groups, and only 13 (38%) of them were in the seven O groups cited by Ørskov et al. Eight of these were included in the four major LT-ST O groups; the remaining five were in the O128 serogroup. Since no LT-ST strains were in the O128 O group, this suggests that there may also

TABLE 2. *O:K:H serotype and enterotoxin type of ETEC strains*

Serotype	No.	Enterotoxin type	Biotype[a]
O4:K98:H$^-$	1	ST	
O6:K15:H16	9	ST-LT	Same[b]
O7:K$^-$:H4[c]	1	ST	
O8:K8:H2	1	ST	
O8:K40:H9	18	ST-LT	Same
O8:K47:H?	1	LT	
O8,O60:K47:H19	1	ST-LT	
O15:K$^-$:H11	1	ST-LT	
O20:K?:H$^-$	1	ST-LT	
O20,O153:K$^-$:H10	2	ST	Same
O20,O153:K$^-$:H12	1	ST	
O25:(K7):H42[d]	2	ST-LT	Same
O29:K$^-$:H21	2	ST	Same
O34:K$^-$:H10	2	ST	Same
O48:K$^-$:H26	1	LT	
O63:K$^-$:H12	1	ST	
O63:K$^-$:H12	1	ST-LT	
O63:K$^-$:H30	1	ST-LT	
O63:K$^-$:H?	1	ST	
O78:K$^-$:(H11)[e]	6	ST-LT	Same
O78:K$^-$(H12)	12	ST-LT	Same
O78:K$^-$:(H12)	5	ST	
O78:K$^-$:H spont. aggl.[f]	2	ST-LT	
O85:K$^-$:H7	2	ST-LT	Same
O85:K$^-$:H spont. aggl.	1	ST	
O96:K$^-$:H19	1	LT	
O114:K$^-$:H21[e]	1	ST	
O114:K$^-$:H49	1	LT	
O115:K$^-$H21	2	ST	Same
O115:K$^-$:H40	5	ST-LT	Same
O115:K$^-$:(H51)	6	ST-LT	Same
O123:K$^-$:H28	1	ST	
O126:K$^-$:H12[e]	1	ST	Same
O126:K:H$^-$	1	ST-LT	
O128:K$^-$:H12[e]	1	ST	
O128:K$^-$:H21	3	ST	Same
O128:K$^-$:H spont. aggl.	1	ST	
O148:K$^-$:H28	1	ST	
O159:K$^-$:H4	1	LT	
OX2:K$^-$:H41	1	LT	
Negative O1-O163: K?:H21	1	ST	
Negative O1-O163: K$^-$:H27	1	ST	
Negative O1-O163: K$^-$:H33	1	ST-LT	
Negative O1-O163: K?:H spont. aggl.	1	ST	
Spont. aggl.:K$^-$: H10[g]	1	ST	
Spont. aggl.:K$^-$: H10[g]	1	ST	

[a] The following sugars were used for biotyping: adonitol, dulcitol, sorbitol, rhamnose, xylose, maltose, salicin, inositol, lactose, sucrose, and sorbose; the lysine and ornithine decarboxylase tests were also used.

[b] Same means that the nine strains had the same biotype.

[c] K$^-$ means that the strain was negative in the two-dimensional electrophoresis test in agarose for acidic K polysaccharide in which Cetavlon was used in the second dimension (15).

[d] Parentheses mean that minus variants (H or K) with the same biotype were found.

[e] These O antigens were described previously with a K antigen of the B type (O78:K80, O114:K90, O126:K71, and O128:K67). Since these K antigens have never been demonstrated serologically or chemically as independent antigens, they have been left out here (12).

[f] H spont. aggl. means that the motile culture was spontaneously agglutinable.

[g] These boiled cultures were spontaneously agglutinable, making O determination impossible.

TABLE 3. *Biotypes of ETEC serotypes*

Test	Serotype							
	O78:K$^-$: (H12) (n = 17)	O115:K$^-$: H40 (n = 5)	O115:K$^-$: (H51) (n = 6)	O128:K$^-$: H21 (n = 3)	O6:K15: (H16) (n = 9)	O8:K40: H9 (n = 18)	O25:K7: H42 (n = 2)	O78:K$^-$: (H11) (n = 6)
Adonitol	−[a]	−	−	−	+	−	−	+
Dulcitol	x	−	+	+[b]	−	+	−	−
Sorbitol	+	+	+	+	+	+	−	D
Xylose	+	+	+	+	+	+	+	−
Rhamnose	+	+	+	+	D	+	+	x[c]
Maltose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Sucrose	+	x	+	+	−	x	−	−
Sorbose	−	−	−	−	−	+	−	−
Ornithine	−	D	−	−	−	+	D	−

[a] Symbols: +, positive on day 1 or 2; −, negative after 30 days; x, late or negative; D, different reactions in different strains.

[b] Positive on days 2 to 7.

[c] Most O78:K$^-$:(H11) strains reported previously (12) were positive on day 2 in the rhamnose test, whereas most of those presented here were positive on day 3.

be certain O serogroups in which only ST *E. coli* may be found, although this may be considerably less common than with LT-ST strains. An insufficient number of LT strains were isolated in this study to draw firm conclusions about these strains. Serotype data from one study in Kenya did suggest that LT strains, like ST strains, were not restricted to a few O groups (16), but in another study done in the same location 1 year later all seven LT-producing strains isolated belonged to serogroups O159:H4 and O159:H34. It was possible, however, that in the latter study the strains were epidemiologically related (15).

In this study we found that except for two O78 strains and one O8,O60 strain, all of the LT-ST *E. coli* belonging to the four major LT-ST O groups were confined to six O:K:H serotypes. Four of these were included in the original list of Ørskov and Ørskov (11); the two additional serotypes were O115:H40 and O115:H51. In addition, we isolated two LT-ST *E. coli* strains with the O25:K7:H42 serotype reported by Ørskov et al. Our finding of five ST strains with the same biotype as the 12 LT-ST O78:K$^-$:(H12) strains indicates that the five strains may have changed from LT-ST to ST strains, as postulated by Evans et al. (2) and suggests that in studies with larger numbers of isolates it would not be surprising to find ST strains belonging to one of the common LT-ST serotypes.

It is not clear why LT-ST strains belong to a more restricted number of O groups and serotypes. Perhaps the larger LT-ST plasmid (4) can be more readily accepted and retained by *E. coli* with specific surface characteristics that include O antigen.

The O serogroups found in this study are similar to those reported by Sack et al. in a study at our hospital of 23 ETEC patients conducted in November 1974 (17). Similar serotype and enterotoxin data are needed from other geographic locations to determine whether our findings are unique to Bangladesh. This information is critical to the development of serological pools for slide agglutination for rapid, simple diagnosis of ETEC disease and for studies of its epidemiology. A study is presently in progress to determine the incidence of serotypes of additional ETEC strains as well as of non-ETEC strains.

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