Relationship of K1 Antigen to Biotype in Clinical Isolates of *Escherichia coli*

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Two hundred and ninety-four isolates of *Escherichia coli*, including 105 from blood cultures, 94 from stools of hospital inpatients, and 96 from rectal cultures of healthy young adults, were biotyped by using the API-20E system and tested for the presence of K1 antigen. The overall frequency of K1 strains was 14.2% and was similar among the three sources. Forty-eight biotypes were observed, but two-thirds of all isolates, including two-thirds of the K1 strains, belonged to only five biotypes. Among the five commonest biotypes, the distribution of K1 strains was nonrandom, since 23 of the 27 K1 strains belonged to only two biotypes. Analysis of the O and H antigens of K1 strains indicated that this correlation of biotype with K1 antigen was due to a restricted number of serovars ("clones") that were repeatedly isolated from the population studied. These serovars included O18:K1:H7, O1:K1:H6, and O16:K1:H6. Although a statistically significant correlation between biotype and K1 antigen was observed, the correlation was not sufficiently great to allow biotyping to be of significant predictive value as a marker for the K1 antigen.

Several investigators have recently examined the relationship of biotype to serovar in *Escherichia coli* (1, 11) and other *Enterobacteriaceae* (6, 8, 9). Most have concluded that these characteristics vary independently; i.e., one serovar may include several biotypes and vice versa.

The relationship of biotype to a specific antigen of clinical interest, such as the K1 antigen of *E. coli*, has not been previously studied. If a relationship were found in which the antigen was present in only a restricted number of biotypes, then biotyping of clinical isolates might have some predictive value as a marker for the antigen. Such a finding could be relevant for the clinical laboratory, since the introduction of miniaturized systems such as API-20E (Analytab Products Inc., New York) presently allows routine biotyping of clinical isolates of *Enterobacteriaceae*.

The K1 capsular antigen appears to be a "virulence factor" for *E. coli*. K1 *E. coli* have been definitely associated with neonatal meningitis (7), childhood pyelonephritis (2), and increased virulence for laboratory animals (10). This report describes a correlation study of K1 antigen and API biotype among clinical isolates of *E. coli* obtained from three different sources.

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MATERIALS AND METHODS

***E. coli* isolates.** A total of 294 strains of *E. coli* were studied. The strains were derived from three sources: (i) 105 isolates from blood cultures of adult patients with bacteremia; (ii) 93 isolates from stool cultures of hospitalized adult patients; and (iii) 96 isolates from rectal cultures of healthy young adult outpatients ("normals") who were being evaluated for venereal disease. All blood and stool culture isolates and 51 of the rectal culture isolates were obtained from patients at the Peter Bent Brigham Hospital, Boston, Mass. The rest of the rectal cultures were obtained at the venereal disease clinic of Newton-Wellesley Hospital, Newton, Mass. Each strain originated from a different patient and was stored for several years in nutrient semisolid agar.

Biotyping. The API-20E card system was used for biotyping. This system includes the following 20 tests: *o*-nitrophenyl- β -D-galactosidase, arginine dihydrolase, lysine and ornithine decarboxylase, citrate utilization, H₂S production, urease, tyrosine deaminase, indole production, acetoin production, gelatinase, and fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, and arabinose. Stock cultures were

streaked on eosin-methylene blue agar (Difco). A triple sugar iron and API-20E cards were inoculated from an isolated colony. The latter inoculation was done according to the manufacturer's instructions. The cards were incubated for 20 h at 36°C. The results of these 20 tests plus an oxidase test were divided into seven groups of three (with the oxidase test last). In each group of three, a positive result of the first test was given a value of 1, the second test a value of 2, and the third test a value of 4. Negative reactions were given a value of zero. The results of each group of three tests were totaled to a single digit (maximum number = 7). Thus a seven-digit profile number was generated, which was located in the API analytic profile index. This index yields the most likely species identification, the estimated frequency of that biotype among all strains of that species, other possible species identifications, and differential tests needed to determine the identity of the strain. This seven-digit API profile number was used as the biotype designation.

All K1 *E. coli* strains were also tested for growth in ammonium-glucose and fermentation of the following carbohydrates by using standard procedures (4): dulcitol, xylose, rhamnose, salicin, sucrose, lactose, and sorbose.

Serotyping. K1 typing was done by immunodiffusion (3). The antigen was a saline bacterial suspension prepared from a Trypticase soy agar plate (BBL) subcultured from the triple sugar iron slant. Antibody globulin was prepared in a horse with glutaraldehyde-fixed group B meningococci (7). The presence of K1 antigen was confirmed by demonstrating a line of identity with a reference strain obtained from the Center for Disease Control, Atlanta, Ga. O and H typing of K1 strains was performed as described (4). All biotyping and serotyping procedures were performed in duplicate for each strain.

RESULTS

The identity of all strains was confirmed as *E. coli* by the API analytic profile index. The

overall frequency of K1 *E. coli* was 42/294 (14.2%). There was no significant difference in frequency of K1 *E. coli* among the three sources studied (14 [13.3%] blood culture isolates, 12 [12.3%] stool culture isolates from hospitalized patients, and 16 [16.6%] rectal culture isolates from "normal" adults).

Identical biotypes were observed on initial duplicate testing for 218 (74%) strains. These strains included 33 (79%) of the 42 K1 strains. The 76 strains that were not reproducibly biotyped on initial testing were equally distributed among the three sources studied. Of these 76 strains, 61 (80%) varied in only one biochemical test, usually melibiose or arabinose fermentation. These 76 strains were restreaked from the stock culture, and two colonies of each strain were individually biotyped. The entire procedure was then repeated. Reproducible biotypes were obtained on all but two strains on this attempt. The two non-biotypable strains were eliminated from analysis. Conventional fermentation testing of the K1 strains was 100% reproducible.

A total of 48 biotypes of *E. coli* were observed. However, five biotypes accounted for approximately two-thirds of all the isolates and two-thirds of the K1 strains (Table 1). These five biotypes were all common ones, as determined by their estimated frequency in the API analytic profile index. However, several equally common biotypes were only sparsely represented by our strains. Among the five commonest biotypes, the major discriminant reactions were fermentation of sucrose, rhamnose, and melibiose.

The distribution of K1 strains among the five common biotypes was not random (Table 2). Of the 27 K1 *E. coli* strains within these five bio-

TABLE 1. Commonest biotypes among the clinical isolates of *E. coli*

API profile (biotype)	Estimated frequency ^a	Observed frequency			
		Blood cultures (n = 105)	Stool cultures (hospitalized patients) (n = 93)	Rectal cultures ("normals") (n = 96)	All sources (n = 294)
5 144 572	1/11 (9) ^b	21 (20) ^c	17 (18)	18 (19)	56 (19)
5 144 552	1/7 (15)	20 (19)	21 (23)	11 (11)	52 (18)
5 044 552	1/17 (6)	14 (13)	8 (9)	19 (20)	41 (14)
5 044 572	1/26 (4)	6 (6)	11 (12)	8 (8)	25 (9)
5 144 512	1/23 (4)	14 (13)	5 (5)	2 (2)	21 (7)
Other ^d		30 (29)	31 (33)	38 (40)	99 (33)

^a Frequency with which *E. coli* isolates are expected to have that particular profile according to the API analytic profile index.

^b Numbers in parentheses are percentages.

^c Percentages of total isolates from that source.

^d Includes 43 separate profiles.

TABLE 2. Frequency of K1 strains among the commonest *E. coli* biotypes

API profile (bio-type)	Blood cultures	Stool cultures (hospitalized patients)	Rectal cultures ("normals")	All sources
5 144 572	0/21	2/17	0/18	2/56 (2) ^a
5 144 552	2/20	6/21	8/11	16/52 (31)
5 044 552	0/14	1/8	1/19	2/41 (5)
5 044 572	0/6	0/11	0/8	0/25 (0)
5 144 512	6/14	0/5	1/2	7/21 (33)
Others	6/30	3/31	6/38	15/99 (15)

^a Numbers in parentheses are percentages.

types, 23 (85%) were found in only two of the biotypes. The difference in distribution of K1 *E. coli* from non-K1 strains is highly significant, whether one compares only strains within the five commonest biotypes ($P < 0.001$, $\chi^2 = 30.9$ with 4 df) or all strains ($P < 0.005$, $\chi^2 = 30.2$ with 5 df).

The O and H serovars of the K1 *E. coli* are given in Table 3. Of 16 strains within the most commonly found biotype, 13 (81%) were of only two serovars (O18ac:K1:H7 and O1:K1:H7). Four (57%) of seven strains within the second most common biotype were of the same serovar (O16:K1:H6), and one other strain was a closely related serovar (O16:K1:H⁻). The 15 strains among the sporadically occurring biotypes were of a wider variety of serovars, including five different O antigens.

This apparent association of biotype with specific serovars was further studied by standard fermentation reactions with a variety of carbohydrates (see Materials and Methods). Of the 42 K1 strains observed in the study, eight were sucrose positive and none were serovar O18ac:K1:H7. Of the 42 strains, 34 were sucrose negative and 11 were serovar O18ac:K1:H7.

There were 12 strains that had identical reactions: dulcitol (+), xylose (+), rhamnose (+), salicin (+), sucrose (-), lactose (+), sorbose (+), and no growth in ammonium-glucose. Eight (67%) of these 12 strains were O18ac:K1:H7, two were spontaneously agglutinable:K1:H7, one was O1:K1:H7, and one was spontaneously agglutinable:K1:H31.

DISCUSSION

This study indicates that there was a correlation of biotype as determined by the API-20E system with the presence of K1 antigen among clinical isolates of *E. coli*. The distribution of K1 strains among the biotypes most commonly observed was definitely nonrandom. Two hypotheses could explain this apparent correlation of biotype with K1 antigen. The first hypothesis is that these two characteristics are "linked," based perhaps on genetic and/or biochemical grounds. The second hypothesis is that there are a restricted number of K1-containing serovars ("clones"), indigenous to the population studied, which were repeatedly isolated. The O and H serovar data showing that most of the K1 strains within the most commonly observed biotypes belonged to only three serovars (O18ac:K1:H7, O1:K1:H7, and O16:K1:H6) strongly favor the latter hypothesis. Robbins et al. (7) have shown that K1 *E. coli* isolated from the cerebrospinal fluid of neonates with meningitis are also restricted in the distribution of O antigens, such that antigens O18ac, O7, O1, and O16 accounted for two-thirds of their strains. The phenomenon of bacterial "cloning" of pathogenic *E. coli* was recently advanced by Ørskov et al. (5) as the explanation for serovar restriction among enterotoxigenic *E. coli* isolated from individuals in many parts of the world.

TABLE 3. O:H serovars among the K1 strains of *E. coli*

API profile (bio-type)	No. of K1 strains	No. of O:H serovars			
		O18ac:H7	O1:H7	O16:H6	Others ^a
5 144 572	2				O83:H4
5 144 552	16	8	5		sp.ag. ^b :H7 (2)
5 044 552	2				sp.ag.:H7
5 044 572	0				
5 144 512	7	1		4	O16:H ⁻
Others	15	2		1	sp.ag.:H ⁻ (3)
					O2:H6
					O75:H7
					O132:H ⁻
					O7:H ⁻ (2)
					O75:H ⁻

^a Number in parentheses indicates the number of strains of that serovar. If no number is listed then only one strain of that serovar was found.

^b sp.ag., Spontaneously agglutinable.

All *E. coli* isolates in this study had been stored for several years. The stability, during prolonged periods of storage, of the K1 antigen and many of the biochemical reactions used for biotyping need further study, and results with freshly isolated strains could differ somewhat. Future studies of K1 antigen and biotype should include such freshly isolated strains and/or strains stored under conditions that would minimize phenotypic changes, e.g., strains that have been lyophilized, or frozen at -70°C .

The original goal of this study was to investigate whether certain biotypes could be identified as markers with high predictive value for K1 antigen. A statistically significant correlation between biotype and K1 antigen was observed, since the majority of K1 strains (23/42) belonged to only two biotypes. However, there were 15 biotypes with at least one K1 strain, indicating that biotyping alone cannot predict the presence of K1 antigen. Furthermore, the frequency of K1 strains was only about one in three among the two biotypes with the greatest number of K1 strains. Therefore, the correlation between biotype and K1 antigen was not sufficiently great to allow biotyping to be of significant predictive value as a marker for the K1 antigen.

The overall frequency of K1 *E. coli* observed in this study (14.2%) is similar to the results of other recent investigations (4, 7). The comparable frequency of K1 strains among isolates from adult patients with serious *E. coli* disease (bacteremia) compared with the frequency in fecal strains from hospitalized adult patients and "normal" adult outpatients has also been previously observed (7). The K1 antigen of *E. coli* thus appears to be associated with disease only in the pediatric age group and not in adults (10).

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