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Comparative Analysis of Plasmids and Some Metabolic Characteristics of *Escherichia coli* K1 from Diseased and Healthy Individuals

RICHARD P. SILVER,* WENDY AARONSON, ANN SUTTON, AND RACHEL SCHNEERSON

Division of Bacterial Products, Bureau of Biologics, Food and Drug Administration, Bethesda, Maryland 20205

All 62 *Escherichia coli* strains possessing the K1 capsular polysaccharide contained plasmid deoxyribonucleic acid, and most (51 of 62) had multiple plasmid species. The incidence of hemolysins, colicins, hemagglutinins for human erythrocytes, and plasmids did not differ among K1 strains isolated from the cerebrospinal fluids of neonates with meningitis or among those strains isolated from the stools of healthy individuals of all ages. There was an association between *E. coli* serotype and the distribution of plasmids, hemolysins, and colicins among the K1 strains. A common plasmid of about 65 megadaltons was found in all of the O18 serotypes; the similarity of these plasmids was confirmed by analysis with the restriction endonuclease *Eco*RI. Plasmids of similar molecular weight were also present in *E. coli* strains of the O7:K1 and O75:K100 serogroups. These data are consistent with the hypothesis that *E. coli* strains of the same serotype may be descendents of a single bacterial clone.

Escherichia coli is the most common cause of neonatal bacterial meningitis, accounting for approximately 40% of all such cases (11, 20). Although antigenically *E. coli* is a complex species, consisting of 164 O (lipopolysaccharide), 103 K (capsular), and 56 H (flagellar) antigens (32), disease isolates possessing the K1 capsular polysaccharide account for about 80% of the cases of *E. coli* neonatal meningitis (33). The K1 capsule is also detected with high frequency in neonatal septicemia without meningitis and in childhood pyelonephritis (14, 19). K1 strains have been found in 20 to 40% of healthy individuals of all ages, including newborns (35). Thus, several variables, including host resistance factors, must be characterized to further define the pathogenic role of the K1 capsular polysaccharide.

Bacterial plasmids confer virulence properties to some *E. coli* strains associated with diarrheal disease. The production of enterotoxins and the ability of the bacteria to colonize the upper small intestine are mediated by transmissible plasmids (10, 12, 30, 40). Production of hemolysins, colicins, and specific adherence factors (23, 43) was observed more frequently among *E. coli* strains isolated from extraintestinal sites than among those strains isolated from the normal flora of healthy individuals (3, 41, 45).

In this study we compared the incidence of plasmids and other properties in *E. coli* K1 strains isolated from neonatal meningitis with the properties of K1 strains isolated from normal flora. In addition, these factors were also analyzed in *E. coli* possessing the K100 antigen, a capsular polysaccharide not associated with human disease.

MATERIALS AND METHODS

Bacterial strains. A total of 45 strains of *E. coli* K1 isolated from the cerebrospinal fluids (CSF) of neonates were studied. The O, K, and H antigens (serotypes) and activities in a neonatal rat model of two K1 strains (C94 and EC3) have been described previously (8). The remaining strains were obtained from the Cooperative Neonatal Meningitis Study (33) and had been stored freeze-dried in 10% skim milk suspensions. A total of 52 strains from the feces of healthy children and adults, 2 strains from urinary tract infections, and 2 laboratory strains (D699, O16) (29) were examined. Urinary and fecal isolates were kindly donated by George H. McCracken, Jr., University of Texas Health Science Center, Dallas, J. C. Parke, Jr., Charlotte Memorial Hospital, Charlotte, N.C., and Suburban Hospital, Bethesda, Md. Eight O75:K100:H5 *E. coli* strains were included in the study. Strain Easter has been described previously (37). Five K100 strains were isolated recently in Texas from infants with *Haemophilus influenzae* type b disease (7). The presence of the K1 and K100 capsular polysaccharides was confirmed by halo formation on Davis minimal medium (Difco Laboratories, Detroit, Mich.) agar containing group B meningococcal and *H. influenzae* type b antisera, respectively (8, 26, 38). K1 isolates consisted of strains from O antigen groups 1, 7, 16, 18, 83, 123, 132, and 156 (see Table 2). A total of 5 isolates agglutinated spontaneously, and the O serogroups of 62 isolates were not determined.

Media. Working cultures were maintained on nu-

trient agar slants. The culture media used were brain heart infusion broth and Trypticase soy broth and agar (BBL Microbiology Systems, Cockeysville, Md.). The antibiotic susceptibility of each isolate was determined by the disk diffusion method (1). Beta-hemolysis was detected after overnight incubation on Columbia blood agar base (Difco) containing 5% sheep erythrocytes. Colonization factor antigen agar has been described previously (6).

Sensitivity to K1-specific bacteriophages. All K1 strains were assayed for sensitivity to five K1-specific bacteriophages (9), which were kindly provided by B. Rowe, Central Public Health Laboratories, London, England. The phage sensitivities of *E. coli* strains were determined by spot tests (22).

Isolation and characterization of plasmid DNA. Plasmid deoxyribonucleic acid (DNA) was isolated by cesium chloride-ethidium bromide equilibrium centrifugation (5). Electrophoresis of purified plasmid DNA on 0.7% agarose (Seakem, Marine Colloids, Rockport, Maine) gels was performed as described previously (21). Digestion of plasmid DNA with restriction endonuclease *Eco*RI (Bethesda Research Laboratories, Rockville, Md.) was done in a solution containing 100 mM tris(hydroxymethyl)-aminomethane (pH 7.4), 50 mM NaCl, and 10 mM $MgCl_2$ at 37°C for 1 h.

Colicin production. Colicin production was detected by the agar overlay method (16). Cells were inoculated into Trypticase soy agar with sterile toothpicks. After overnight growth at 37°C, cultures were treated with chloroform for 30 min. Plates were air dried for at least 30 min and overlayed with soft agar (0.6% nutrient agar) containing *E. coli* strain K12 with or without the colicin V plasmid. Zones of inhibition were observed after overnight incubation at 37°C. Strains which inhibited K12 but not K12 containing colicin were considered to be colicin V producers.

Hemagglutination. Hemagglutination (HA) reactions were performed as described by Evans et al. (6). Human type A blood was collected in 3.8% sodium citrate. Cells were washed three times in phosphate-buffered saline and resuspended in phosphate-buffered saline or phosphate-buffered saline containing 0.05% D-mannose to a concentration of 3% (vol/vol). Bacteria were grown overnight on colonization factor antigen agar and suspended in 20 μl of blood on a glass slide at room temperature. Macroscopic HA was recorded within 1 min. If no HA was observed, the slide was placed on ice for 5 min with intermittent rotation. Reactions were recorded as 4+ (immediate and complete), 3+ (immediate or delayed, more than 50% clumping), 2+ (immediate or delayed, less than 50% clumping), 1+ (slight but visible clumping), or negative. All positive reactions were also tested for HA in the presence of mannose.

RESULTS

Plasmids in encapsulated *E. coli*. All 62 *E. coli* K1 strains examined contained plasmid DNA, and most (51 of 62) possessed multiple plasmid species. The number of plasmids per strain ranged from 1 to 11, with an average of about 4. Figure 1 shows the heterogeneity in the number of plasmids observed. These plasmids ranged from about 0.3 to 60 megadaltons (Mdal). In general, *E. coli* K1 plasmids seemed to fall within two size classes, those equal to or less than 5 Mdal or those greater than 25 Mdal. All *E. coli* K1 isolates contained at least one high-molecular-weight plasmid. The incidence of plasmids did not differ between disease isolates and isolates from healthy individuals, nor was there evidence of a plasmid common to the disease isolates. All eight *E. coli* K100 isolates examined also contained multiple species of plasmid DNA (Fig. 1, slot e).

Hemolysin and colicin production and hemagglutinating properties of *E. coli* isolated from CSF and from normal flora. Table 1 compares the production of colicins and hemolysins and the HA of human erythrocytes in 45 CSF isolates of *E. coli* K1 and 52 strains isolated from the feces of healthy children and adults. There were no significant differences between these two groups of organisms; approximately 50% of all isolates, irrespective of source, produced colicins, were hemolytic, and hemagglutinated human erythrocytes. A total of 86% of the HA reactions were resistant to mannose; this included 71% of the CSF isolates and 96% of the fecal isolates ($P < 0.05$). A total of 20% of all strains contained R plasmids. There were no differences between strains isolated from healthy children and those from adults (data not shown). Of 22 colicin-producing strains from CSF, 6 produced colicin V, and 1 of 25 strains from normal flora produced colicin ($P > 0.05$). Of the 97 K1 strains, 87 were sensitive to one or more of the five K1 bacteriophages.

The relationships between the properties described in Table 1 and the serotypes of the *E. coli* strains are shown in Table 2. *E. coli* O18 and O7 were the most common O serotypes among the *E. coli* K1 strains assayed; 80% of O18:K1 isolates were hemolytic, whereas none of the O7:K1 isolates produced hemolysin. In contrast, 88% of the O7:K1 strains were colicinogenic, but only 13% of the O18 isolates produced colicin ($P < 0.01$). The O18 strains averaged less than two plasmids per cell, whereas more than seven plasmids per cell were found in O7 isolates ($P < 0.001$). All eight *E. coli* K100 isolates examined were O75 (Table 2). A total of 50% of the K100 strains were colicinogenic, none were hemolytic, and two of eight strains hemagglutinated human erythrocytes.

Plasmids from *E. coli* O18:K1 and O75:K100. Figure 2 compares the plasmids from eight independently isolated O18:K1 strains. These strains were from different geographic regions and included five isolates from CSF, one urinary tract isolate, and two normal flora. In

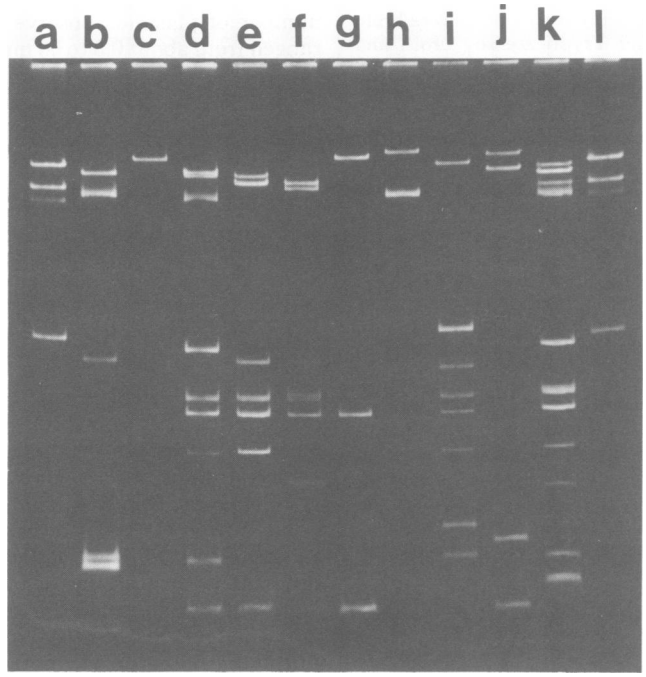

FIG. 1. *Electrophoresis in a 0.7% agarose gel of purified plasmid DNA from encapsulated E. coli. Slots a and l contained the molecular weight standards pSR19 (50 Mdal), RP4 (34 Mdal), S-a (25 Mdal), and RSF1050 (5 Mdal). Slot b, RS170 (O7:K1:H-); slot c, RS215 (O18:K1:H7); slot d, RS168 (O1:K1:H-); slot e, RS508 (O75:K100:H5); slot f, RS176 (O7:K1:H-); slot g, RS167 (O16:K1:H6); slot h, RS202 (O83:K1:H-); slot i, RS185 (not serotyped); slot j, RS181 (spontaneous agglutinating K1); slot k, RS231 (not serotyped).*

TABLE 1. *Properties of E. coli K1 isolates*

Source of strains	No. of strains tested	No. with the following properties:					
		Antibiotic resistance	Colicin positive	Colicin V positive	Hemolysin	HAh[a]	Plasmid containing
CSF	45	12 (27)[b]	22 (49)	6 (13)	13 (29)	21 (47)[c]	45 (100)
Feces	52	9 (17)	25 (49)	1 (2)	28 (54)	28 (54)[d]	13 (100)[e]

[a] HAh, HA positive for human erythrocytes.
[b] Numbers in parentheses are percentages.
[c] Of 21 strains, 15 (71%) were mannose resistant.
[d] Of 28 strains, 27 (96%) were mannose resistant.
[e] Thirteen of the fifty-two strains were analyzed for plasmid DNA.

contrast to the heterogeneity observed among K1 strains of different O and H serotypes, the plasmids within this particular serotype were similar. A plasmid of about 65 Mdal was detected in each strain. Plasmids of similar size were also observed in the O7:K1 serogroup (data not shown).

Figure 3 shows that the *Eco*RI cleavage patterns of these common O18:K1 plasmids were very similar and contained many common bands. The plasmids isolated from strains RS228 and RS215 (Fig. 3, slots d and e) were identical. The former strain was isolated from a healthy child in Albany, N.Y. in 1978, whereas the latter came from the CSF of a neonate in Alabama in 1973.

The plasmids from O75:K100:H5 *E. coli* strains are shown in Fig. 4. The K100 strains also contain multiple plasmid species, and many plasmids are common to most strains. Strain Easter (Fig. 4, slot b) was isolated in 1971 from the stools of an asymptomatic infant in a chronic care nursery and does not contain high-molecular-weight species (37). It does have the smaller plasmids common to all of the K100 isolates. Strains RS505 through RS509 (Fig. 4, slots e through i) were isolated recently from infants with *H. influenzae* type b disease (7). Strains

TABLE 2. *Properties of E. coli according to serotype*

Serotype of strain	No. tested	No. with the following properties:				No. of plasmids per cell
		Antibiotic resistance	Colicin positive	Hemolysin	HAh[a]	
O18:K1	15	5 (33)[b]	2 (13)	12 (80)	7 (47)	1.6
O7:K1	8	2 (25)	7 (88)	0	4 (50)	7.1
O1:K1	3	0	3 (100)	0	1 (33)	5.0
O16:K1	3	0	2 (67)	0	2 (67)	3.0
O83:K1	2	2 (100)	2 (100)	0	0	2.5
Sp. agg.:K1[c]	5	1 (20)	2 (40)	1 (20)	2 (40)	3.0
Other K1[d]	26	5 (19)	10 (38)	8 (31)	14 (54)	4.3
O75:K100	8	5 (63)	4 (50)	0	2 (25)	5.8

[a] HAh, HA positive for human erythrocytes.
[b] Numbers in parentheses are percentages.
[c] Sp. agg., Spontaneous agglutinating.
[d] Includes O132, O123, O156, and 23 other K1 strains not serotyped.

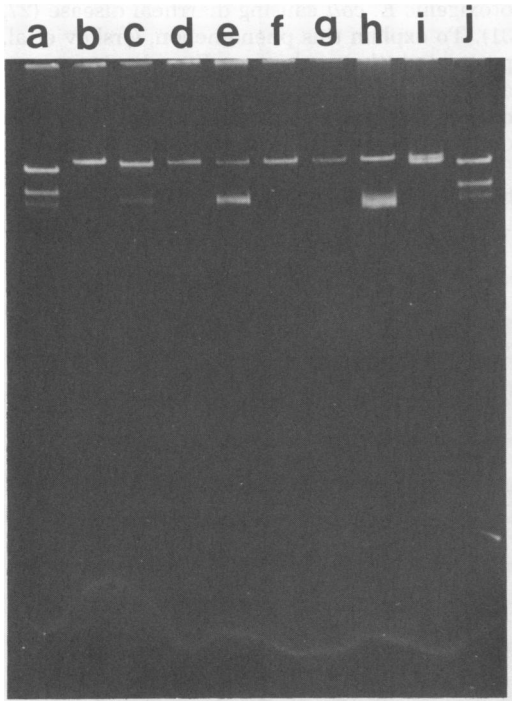

FIG. 2. *Electrophoresis in a 0.7% agarose gel of E. coli plasmid DNA from O18:K1 isolates. Slots a and j contained the molecular weight standards pSR19 (50 Mdal), RP4 (34 Mdal), and S-a (25 Mdal). Slot b, RS229; slot c, RS218; slot d, RS228; slot e, RS203; slot f, RS215; slot g, RS195; slot h, RS162; slot i, RS226.*

RS507 through RS509 are ampicillin resistant and have identical plasmid patterns. RS506 is ampicillin sensitive and has lost one plasmid species.

DISCUSSION

All of the strains examined contained plasmid DNA, illustrating the high incidence of plasmids in *E. coli* strains from both intestinal and extraintestinal sources (10, 42). A majority harbored more than one distinct plasmid species. The functions of most of these plasmids cannot be accounted for by known determinants, such as antibiotic resistance, colicin production, or virulence properties. The incidence of plasmids in *E. coli* strains from urinary tract infections, as reported by Minshew et al. (24), was about 60%. However, these workers found no association between plasmids and the virulence properties of their urinary tract isolates. The colicin V plasmid has been reported to be related to the invasiveness of *E. coli* strains for domestic animals (41). Our results and those of Minshew et al. (23) indicate that the colicin V plasmid is not significant in human disease.

Analysis by restriction endonucleases provides a sensitive method for determining relatedness between plasmids (44). *Eco*RI digestions of the common O18 plasmids showed that they are identical or closely related. It is unlikely that the same plasmid was transferred independently to other O18:K1 strains. Our hypothesis is that these strains were derived from a single *E. coli* clone.

A total of 50% of the K1 strains agglutinated human erythrocytes; this property was not related to the serotypes of the organisms. This is probably a low estimate of the pilus-like adherence structures since hemagglutinins for other animal species were not sought (4, 6). There is evidence that pili play an important role in the adhesion of bacteria to mucosal surfaces (12, 34) and that this property may be directly related to virulence (39, 43). HA reactions mediated by common pili of *E. coli* are inhibited by D-mannose (4). In contrast, HA reactions by other surface structures associated with virulence, such as the K88 antigen or gonococcal pili, are mannose resistant (2, 13). A total of 86% of the HAs were mannose resistant, suggesting that

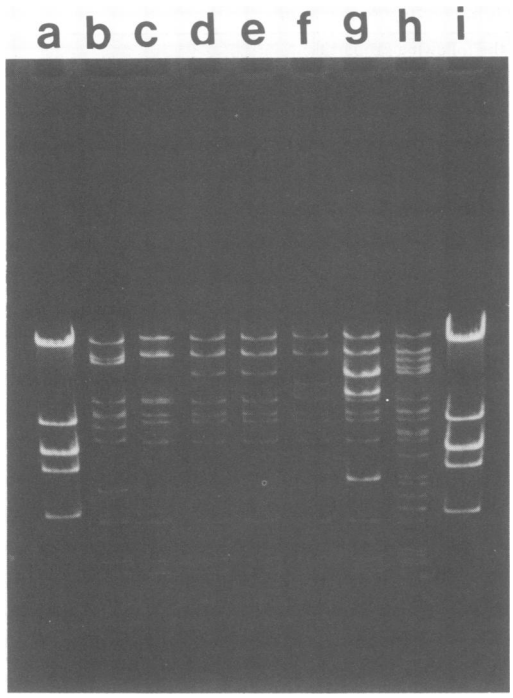

FIG. 3. *Electrophoresis in a 0.7% agarose gel of EcoRI-digested E. coli plasmid DNA from O18:K1 isolates. Slots a and i contained λ DNA fragments of 21.8, 7.6, 5.5, 4.8, and 3.4 kilobases. Slot b, RS229; slot c, RS218; slot d, RS228; slot e, RS215; slot f, RS195; slot g, RS162; slot h, RS226.*

surface structures other than the common pili mediate the adherence of the K1 strains to the intestinal mucosa. The slight statistical difference ($P < 0.05$) in mannose resistance between hemagglutinins from *E. coli* K1 strains from CSF (71%) and hemagglutinins from the strains from stools of healthy individuals (96%) is under study. Further investigations on the surface structures of *E. coli* K1 strains and their roles in pathogenesis are planned.

K1 strains isolated from CSF and from stools of healthy individuals did not differ in their production of hemolysins or colicins, in their ability to agglutinate human erythrocytes, or in their plasmid contents. These results are consistent with epidemiological data showing that neonatal disease isolates are derived from the intestinal flora of healthy individuals (35). However, production of colicins and hemolysins and the plasmid contents of K1 strains were homogeneous when grouped by serotype, in contrast to the heterogeneity of these characteristics when studied in a random population (Table 2). A similar correlation between serotype and the biochemical properties of K1 isolates from both diseased and healthy individuals was reported by Myerowitz et al. (25). K1 strains with the most commonly observed biotypes belonged to only three serotypes: O18:K1:H7, O1:K1:H7, and O16:K1:H6 (25).

It has been shown that invasive *E. coli* strains are associated with only a few serotypes (26, 32). Of the more than 100 K antigens, 5 account for most upper urinary tract disease (15); 80% of the *E. coli* strains isolated from the urine of children with acute pyelonephritis belong to eight common O serogroups (17, 18). The distribution of somatic O antigens among K1 strains is not random; relatively few serotypes predominate. Four O antigens (O18ac, O7, O1, and O16) account for two-thirds of the K1 strains isolated from the CSF of neonates (35). Certain O:H serotypes are frequently associated with enterotoxigenic *E. coli* causing diarrheal disease (27, 31). To explain this phenomenon, Ørskov et al. postulated that only few *E. coli* strains are adapted for invasiveness in humans and that disease isolates of the same serotype from a defined disease may be descendants of one or a few clones (27). Pathogenicity of *E. coli* may not be directly related to the surface antigens, but the serotype may identify those clones adapted

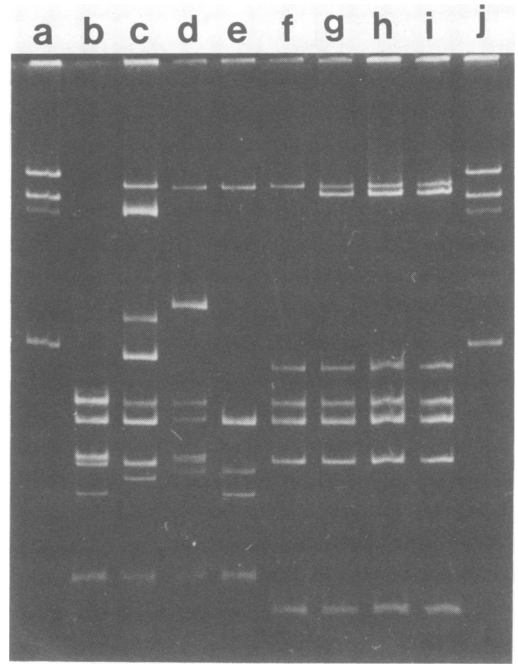

FIG. 4. *Electrophoresis in a 0.7% agarose gel of E. coli plasmid DNA from O75:K100:H5 isolates. Slots a and j contained the molecular weight standards pSR19 (50 Mdal), RP4 (34 Mdal), S-a (25 Mdal), and RSF1050 (5 Mdal). Slot b, Strain Easter; slot c, RS502; slot d, RS503; slot e, RS505; slot f, RS506; slot g, RS507; slot h, RS508; slot i, RS509.*

to synthesize certain macromolecules necessary to cause human disease. Certain O:H serotypes that cause diarrheal disease, for instance, may be especially adapted to carry the plasmids needed to colonize and adhere to the mucous membranes of the small intestine, produce a protein toxin, and therefore cause diarrheal disease. Our results are consistent with this clonal hypothesis. One possibility raised by this theory is that the K1 capsular polysaccharide may not be a virulence factor but may be only related to as yet undefined characteristics of the disease isolates. This seems unlikely since there is much evidence that the K1 capsular polysaccharide confers the property of invasiveness to *E. coli* (20, 28, 33, 36, 46). Other bacterial properties associated with invasiveness, such as iron binding, await definition of their roles in human infectious disease.

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