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Lipopolysaccharide, Capsule, and Fimbriae as Virulence Factors Among O1, O7, O16, O18, or O75 and K1, K5, or K100 *Escherichia coli*

B. KUSECEK,[1] H. WLOCH,[1] A. MERCER,[1] V. VAISÄNEN,[2] G. PLUSCHKE,[1] T, KORHONEN,[2] AND M. ACHTMAN[1]*

Max-Planck-Institut für molekulare Genetik, D-1000 Berlin 33, Germany[1] and Department of General Microbiology, University of Helsinki, Finland[2]

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K1, K5, and K100 *Escherichia coli* isolates of the lipopolysaccharide antigen types O1, O7, O16, O18, or O75, which had formerly been assigned to clonal groupings were compared with K? *E. coli* isolates and with laboratory-derived mutants defective in capsule or lipopolysaccharide synthesis. The amount of K1 capsule, the length distribution of the lipopolysaccharide, and the expression of type I and P fimbriae were determined. The clonal groupings were uniform with regard to these properties within each group but different from each other. Many of the K? strains differed from the clonal representatives. The results are interpreted with regard to the different diseases caused by each of these bacterial groups.

Escherichia coli strains of the K1 capsular serotype represent 80% of all *E. coli* strains isolated from cases of meningitis in newborns and are also frequently isolated from fecal samples of healthy persons as well as from patients with urinary tract infection and sepsis (23, 24). Approximately one-half of K1 isolates are of the O1, O7, or O18 lipopolysaccharide (LPS) antigen type. An analysis of published isolation data revealed that these three serotypes were all common among isolates from cases of bacteremia, but that O1:K1 strains were rarely isolated from meningitis in newborns and that O18:K1 strains were rarely isolated from urinary tract infections (22). O18:K5 bacteria are frequently isolated from urinary tract infections (9) and rarely from newborns with meningitis (23). O75:K100 bacteria are not uncommon among fecal isolates, but rarely cause disease (25, 26).

Bacteria of these serotypes were tested for the ability to colonize the gut and to invade the bloodstream after feeding of newborn rats (22). O7:K1 and O18:K1 strains were efficient at colonization and, with only few exceptions, at invasion of the bloodstream to yield bacteremia. These bacteria seemed to multiply directly in the rat bloodstream and were resistant to the bactericidal effects of rat serum in vitro (21, 22). In contrast, O1:K1, O18:K5, and O75:K100 isolates were capable of colonization, but not of invasion (22). A more detailed analysis of the infection process revealed that although O1:K1 bacteria could penetrate the gut mucosal barriers and do appear in the mesenteric lymph nodes, they were incapable of producing high levels of bacteremia (22). These bacteria were also sensitive to the bactericidal effects of rat serum, as were the O18:K5 and O75:K100 isolates (G. Pluschke and M. Achtman, submitted for publication).

The same bacterial groups were also analyzed with respect to their major outer membrane proteins, biotype, and other properties (2). Three distinct, serotype-associated bacterial clones were recognized to which most O7:K1, O16:K1, and O75:K100 bacteria could be assigned. Most O18:K1 bacteria were assigned to one of two distinct (sub)clones, and most O18:K5 bacteria were assigned to two other (sub)clones. Most O1:K1 bacteria were assigned to one of two clones that differed markedly from each other; one of these two O1:K1 clones closely resembled one of the O18:K1 (sub)clones in all respects except O serotype. The close resemblance of these O1:K1 and O18:K1 bacteria contrasts with their marked differences in disease specificity.

The basis of the clone-specific differences in virulence might be found in cell surface structures such as LPS, capsule, and fimbriae. In this report we present an analysis of the amount of K1 capsular polysaccharide, the amount, length, and nature of O1, O7, O16, O18, and O75 LPS and the expression (or not) of P and type I fimbriae on members of these various serotype groups.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. Many of the bacterial strains used here have been described previously (2). These strains are listed together with the properties measured here in Table 1. In addition several mutant strains were analyzed which were isolated after transposition mutagenesis by Tn*10*, arose spontaneously during storage or were selected as being bacteriophage resistant. The details of their isolation are as follows.

Mutants A26, A67, A487, A488, A691, and A717, which synthesize less K1 capsule than normal, were isolated after spotting bacteriophages on a lawn of bacteria in soft agar. Colonies growing within the spot were streaked to single cells on antiserum agar plates containing horse 46 anti-*Neisseria meningitidis* group B serum (24). Colonies not showing an antibody precipitation halo were tested further. K1 capsule-specific (8) bacteriophages D and E were obtained from Bernard Rowe via R. P. Silver. Bacteriophages BP1 (K1 capsule specific) and BP156 (specificity unknown) were isolated from the Berlin sewage system.

In three cases, storage of smooth strains as frozen glycerol cultures at −30°C resulted in the selective survival of LPS-deficient mutants that were recognized because they did not agglutinate with rabbit anti-O antiserum. In the case of A467, both smooth and rough variants were isolated from the same glycerol culture, whereas for strains 29 and 194, the smooth parent strain is no longer available. Strain 113, which

TABLE 1. Detailed properties of the wild-type strains tested

Sequential no.[a]	Serotype	Membrane protein pattern[b]	Capsule (μg/ml)	LPS[c]	Fimbriae	
					P	I
1	O1:K1	5	22.2 ± 0.6	O1:K1/5	−	−
2	O1:K1	5	21.5 ± 0.8	O1:K1/5	−	−
3	O1:K1	5	8.3 ± 2.1	O1:K1/5	−	−
5	O1:K1	5	23.7 ± 0.3	O1:K1/5	−	+
7	O1:K1	5	24.0 ± 0.6	O1:K1/5	+	+
9	O1:K1	5	26.1 ± 1.1	O1:K1/5	+	+
10	O1:K1	5	20.3 ± 1.3	O1:K1/5	−	−
11	O1:K1	5	20.7 ± 1.2	O1:K1/5	−	−
13	O1:K1	5	25.0 ± 1.2	O1:K1/5	−	−
15	O1:K1	5	20.1 ± 1.3	O1:K1/5	−	−
16	O1:K1	5	25.1 ± 2.0	O1:K1/5	−	−
18	O1:K1	5	24.0 ± 1.5	O1:K1/5	−	+
19	O1:K1	5	22.7 ± 0.1	O1:K1/5	−	−
20	O1:K1	5	21.9 ± 0.7	O1:K1/5	−	+
22	O1:K1	3	19.2	Unique	−	+
25	O1:K1	5	14.2 ± 1.2	O1:K1/5	−	−
26	O1:K1	5	25.0 ± 0.6	O1:K1/5	+	+
27	O1:K1	9	15.4 ± 1.4	O1:K1/9	+	(+)
28	O1:K1	9	16.4 ± 3.7	Fig. 4/8	+	−
29	O1:K1	9	13.3 ± 1.6	Fig. 3/10	+	+
30	O1:K1	9	17.6 ± 0.9	Fig. 4/6	+	+
34	O1:K1	9	16.7 ± 4.3	O1:K1/9	−	+
35	O1:K1	9	19.9 ± 4.4	O1:K1/9	+	+
38	O1:K1	9	19.0 ± 2.6	O1:K1/9	+	+
40	O1:K1	9	19.9 ± 2.3	O1:K1/9	+	+
41	O1:K1	9	18.8 ± 3.1	O1:K1/9	+	+
42	O1:K1	9	17.1 ± 1.0	O1:K1/9	+	+
44	O1:K1	9	17.9 ± 1.5	O1:K1/9	+	+
46	O1:K1	9	18.9 ± 1.2	O1:K1/9	+	+
48	O1:K1	9	18.1 ± 1.4	O1:K1/9	+	+
50	O1:K1	9	16.8 ± 0.5	O1:K1/9	+	+
52	O1:K1	9	19.2 ± 0.8	O1:K1/9	+	+
53	O1:K1	9	18.7 ± 0.1	O1:K1/9	+	(+)
55	O1:K1	9	17.0 ± 0.9	O1:K1/9	+	+
57	O1:K1	6	19.9 ± 0.2	O1:K1/9	+	+
58	O1:K1	6	16.2	O1:K1/9	+	+
59	O1:non K1	2		Unique	−[d]	+
60	O1:non K1	23		Unique	−	+
61	O1:non K1	23		Unique	−	+
62	O1:non K1	26		O1:K1/9	−	+
63	O18:K1	9	15.0 ± 1.8	O18:K1	−	+
64	O18:K1	9	16.7 ± 1.2	O18:K1	−	+
65	O18:K1	9	15.9 ± 2.5	O18:K1	−	+
70	O18:K1	9	18.2 ± 1.7	O18:K1	−	+
71	O18:K1	9	16.4 ± 0.4	O18:K1	−	+
72	O18:K1	9	16.8 ± 2.1	O18:K1	−	+
74	O18:K1	9	16.7 ± 0.1	O18:K1	−	+
76	O18:K1	9	17.0 ± 0.1	O18:K1	−	+
78	O18:K1	9	11.7 ± 0.7	O18:K1	−	+
80	O18:K1	9	16.7 ± 0.4	O18:K1	−	+
89	O18:K1	9	16.9 ± 1.1	O18:K1	−	+
96	O18:K1	6	16.1 ± 0.6	O18:K1	−	+
99	O18:K1	6	18.3 ± 0.5	O18:K1	−	+
101	O18:K1	6	16.6 ± 0.4	O18:K1	−	+
102	O18:K1	6	17.2 ± 2.9	O18:K1	−[d]	+
103	O18:K1	6	14.9 ± 0.1	O18:K1	−	+
105	O18:K1	6	14.9 ± 0.1	O18:K1	−	+
107	O18:K1	6	15.8 ± 0.3	O18:K1	−	+
108	O18:K1	6	4.2	O18:K1	−[d]	+
111	O18:K1	6	15.4 ± 1.1	O18:K1	−	+
113	O18:K1	6	10.8 ± 1.5	Fig. 3/7	−[d]	+
117	O18:K1	10	23.2	Fig. 3/13	−	+
118	O18:K1	17	Not done	O18:K1	+	+
119	O18:K5	6		Unique	+	+
120	O18:K5	18		O18:K5/18	+	+
121	O18:K5	18		O18:K5/18	+	+
122	O18:K5	18		O18:K5/18	+	+
123	O18:K5	18		O18:K5/18	+	+
124	O18:K5	18		O18:K5/18	+	+

TABLE 1—Continued

Sequential no.[a]	Serotype	Membrane protein pattern[b]	Capsule (μg/ml)	LPS[c]	Fimbriae	
					P	I
125	O18:K5	18		O18:K5/18	+	+
126	O18:K5	11		O18:K1	+	+
127	O18:K5	11		O18:K1	+	+
128	O18:K5	11		O18:K1	+	+
129	O18:K5	11		O18:K1	+	+
130	O18:K5	11		O18:K1	+	+
131	O18:K5	11		O18:K1	+	+
132	O18:K5	11		O18:K1	+	+
133	O18:K5	11		O18:K1	+	−
134	O18:K5	11		O18:K1	+	+
135	O18:K5	11		O18:K1	+	+
136	O18:K5	11		O18:K1	+	+
137	O18:K5	18		O18:K5/18	+	+
138	O18:K?	11		O18:K1	+[d]	+
139	O18:K?	20		Unique	−	+
140	O18:K?	19		Unique	−	−
149	O18:K⁻	22		Unique	−	+
150	O18:K?	21		O18:K1	−[d]	+
151	O7:K1	3	16.6 ± 1.1	O7:K1	+	+
152	O7:K1	3	16.7 ± 0.9	O7:K1	+	+
156	O7:K1	3	15.7 ± 0.8	O7:K1	+	+
157	O7:K1	3	16.3 ± 0.1	O7:K1	+	+
163	O7:K1	3	18.6 ± 0.8	O7:K1	+	+
165	O7:K1	3	18.3 ± 3.0	O7:K1	+	+
168	O7:K1	3	17.6 ± 0.6	O7:K1	+	+
172	O7:K1	3	18.3 ± 1.0	O7:K1	+	+
174	O7:K1	3	13.8 ± 3.7	Fig. 4/3	+	+
176	O7:K1	3	18.7 ± 0.7	O7:K1	+	+
180	O7:K1	23	15.1 ± 0.1	O7:K1	+	+
185	O7:K1	3	14.3 ± 1.0	O7:K1	+	+
189	O7:K1	3	23.8 ± 1.6	O7:K1	−[d]	+
190	O7:K1	16	18.0	O7:K1	+	+
191	O7:K1	24	7.2	Fig. 1/4	−	+
192	O7:K1	15	14.7	O7:K1	+	+
193	O7:K⁻	3		O7:K1	+	+
194	O7:K⁻	13		Fig. 3/11	−	−
195	O7:K?	7		O7:K1	−	+
196	O7:K?	23		Unique	−	+
197	O7:K?	27		Unique	+	+
199	O7:K?	28		Unique	+	+
201	O16:K1	12	16.0 ± 0.6	O16:K1	+	−
202	O16:K1	12	19.4 ± 1.9	Unique	+	(+)
203	O16:K1	12	15.9 ± 0.5	O16:K1	+	+
204	O16:K1	12	23.7 ± 2.4	O16:K1	+	+
205	O16:K1	12	20.1 ± 3.1	O16:K1	+	+
206	O16:K1	12	16.6 ± 1.1	O16:K1	+	−
207	O16:K1	12	22.7 ± 3.6	O16:K1	+	+
208	O16:K1	12	18.3 ± 1.2	O16:K1	+	+
209	O16:K1	12	17.8 ± 1.1	O16:K1	+	+
210	O16:K1	12	19.1 ± 0.1	O16:K1	+	+
211	O16:K1	12	10.6 ± 0.9	O16:K1	+	+
212	O16:K1	12	16.7 ± 1.7	O16:K1	+	+
213	O16:K1	12	O-ac+[e]	O16:K1	+	+
214	O16:K⁻	25		O16:K1	−	+
215	O75:K100	11		O75:K100	−	+
216	O75:K100	11		O75:K100	−[d]	−
217	O75:K100	11		O75:K100	−	+
218	O75:K100	11		O75:K100	−	+
219	O75:K100	11		O75:K100	−	+
220	O75:K100	11		O75:K100	−	+
221	O75:K100	11		O75:K100	−[d]	+

[a] These numbers correspond to the sequential numbers in reference 2.
[b] Major outer membrane protein pattern on SDS-PAGE as defined in reference 2.
[c] The LPS designation for most strains refers to the standard patterns characteristic of the clonal members. Exceptional strains are listed as unique (to be described elsewhere) or according to the figure/track in which they are shown.
[d] These strains make X fimbriae, which can agglutinate p̄ erythrocytes.
[e] O-ac+, O-acetyl+.

remains O agglutinable despite LPS alterations, was recognized during routine screening of supposedly smooth strains. A717, which has the same phenotype as strain 113, was isolated after selection with bacteriophage BP156. Two SpAg:K1 (spontaneously agglutinating) strains (A1317, A1318) were supplied by P. Gemski, Walter Reed Army Institute of Research, Bethesda, Md., and have been described elsewhere (4).

Transposon mutagenesis. The O18:K1 strain RS228 (sequential no. 102 in reference 2) was chosen to generate a bank of transposon-induced mutants for a genetic analysis of properties related to virulence. First a mutant carrying an *lacZ* mutation was isolated after mutagenesis with nitrosoguanidine in the presence of chloramphenicol by the method of Sklar (27). This strain, A802, resembles its parent in all other properties tested, including virulence, protein pattern, nutritional requirements, hemolysin production, biotype, and that it carries a single, FI-incompatible plasmid.

The plasmids pBE306 (Fts114::Tn*5* *lac*) and pBE307 (Fts114::Tn*10* *lac*) were generated in E. coli K12 by using λ::Tn*5* (λ*b*221 *c*I857 Pam802 rex::Tn*5*) (16) and λ::Tn*10* (λ55:*b*221 *c*III167::Tn*10* *c*I857 Oam29) (11) as described previously (16). These plasmids remain *lac*$^+$ and are readily lost after growth at 42°C due to the *ts*114 mutation. Plasmid pBE306 was transferred by conjugation to strain A802 at 30°C, resulting in the kanamycin-resistant, lactose-fermenting strain A817. A817 had lost the original FI-incompatible plasmid due to incompatibility with pBE306. A817 was grown at 42°C overnight, and a Lac$^-$ Kan^s variant, called A886, which is plasmid free (A. Mercer, G. Morelli, M. Heuzenroeder, M. Kamke, and M. Achtman, manuscript in preparation), was isolated. pBE307 was introduced by conjugation as above to yield the Lac$^+$ tetracycline-resistant strain A1224.

Single colonies of A1224 were inoculated into 2-ml volumes of L broth containing 0.01% sodium dodecyl sulfate (inhibits conjugation [1]). These cultures were incubated overnight at 42°C with vigorous aeration and were streaked to single cells on eosin-methylene blue agar plates containing lactose and tetracycline. Two-thirds of the cultures yielded at least one Lac$^-$ colony. Only one colony was used per culture, and 1,000 of these colonies were purified by single-colony isolation and frozen in Microtiter wells at −30°C (5). Bacteria derived from these colonies were screened for lack of agglutination by rabbit anti-O18 serum. Four mutants, A463 through A466, were found among the 1,000 cultures. The LPS of strains A463 through A465 is described here. A466 was similar to A463. Mutants affected in other properties will be described elsewhere.

Media and chemicals. The sources and composition of most media and chemicals were as described previously (2). Barbital buffer consisting of 5.6 g of diethylbarbituric acid, 11.1 g of Tris, 134 mg of calcium lactate, and 163 mg sodium azide per liter was adjusted to pH 8.6 with HCl. The following chemicals (sources) were also used: lysozyme (Serva Feinbiochemica, Heidelberg, Germany), calf thymus DNA (Boehringer-Mannheim, Germany), urea (ultra-pure, no. 5505; Bethesda Research Laboratories, Rockville, Md.), agarose (no. A-6013; Sigma Chemical Co., St. Louis, Mo.), *N*-acetyl neuraminic acid (no. N-2388; Sigma), and polyethylene glycol 6000 (no. 807491, Merck-Schuchardt, Hohenbrunn, Germany). Horse 46 antiserum was the generous gift of J. B. Robbins (Food and Drug Administration, Bethesda, Md.). $^{33}PO_4^-$ (carrier free) was from New England Nuclear Corp., Dreieich, Germany.

Sample preparations for LPS analysis. Silver nitrate staining was performed after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of membrane samples prepared by sonication, Sarkosyl extraction, and centrifugation as described previously (2).

For experiments involving autoradiography after SDS-PAGE, ^{33}P-labeled membranes were prepared as follows. Cells were grown in 10 ml of low-phosphate medium (6) at 37°C with aeration. Exponential-phase cells were centrifuged, suspended in 0.1 volume of fresh medium (2×10^9 cells per ml), and incubated for 40 min at 37°C with aeration in the presence of 35 μCi of $^{33}PO_4^-$. The bacteria were centrifuged, suspended in 10 ml of 10 mM Tris buffer (pH 8.0), and broken by sonication at 0°C (80 s at 50% cycle, Branson cell disruptor B-30; Branson Sonic Power, Danbury, Conn.). Unbroken cells were removed by low-speed centrifugation (10 min, 5,000 rpm, Sorvall SM24 rotor, 4°C), and the membranes were pelleted by centrifugation (60 min, 20,000 rpm) and suspended in 50 μl of sample buffer.

Purification procedures. Phospholipids were extracted from ^{33}P-labeled cells (strain RS228) by the method of Ames (3). LPS was isolated from strains A53 (sequential no. 64 in reference 2), A76 (no. 165), and A110 (no. 41) by the hot phenol water method of Westphal and Jann (34).

K1 capsular polysaccharide was purified from strain A23 (no. 3, original name K235) by the method of Gotschlich et al. (7), except that repeated extractions with chloroform-butanol were also performed by the method of Kaijser (10). K1 polysaccharide (45-mg) was obtained from 15 liter of bacterial culture. Analysis by the resorcinol-HCl method of Svennerholm (30), with pure *N*-acetylneuraminic acid as the standard, revealed that over 99% of the purified polysaccharide dry weight could be accounted for as sialic acid. Nucleic acid contamination was tested by fluorescence in the presence of ethidium bromide, with calf thymus DNA as the standard. Approximately 1% of the dry weight of the purified material could be accounted for as nucleic acid contamination. Protein contamination, estimated by the method of McKnight (17) with lysozyme as the standard, was less than 1% (wt/wt). LPS contamination, determined by the *Limulus* lysate method (29) with purified O1 LPS as the standard, was less than 1% (wt/wt).

Sample preparation for capsule measurements. The method of Stevens et al. (28) was modified as follows. Cells were grown in 10-ml volumes of L broth at 37°C to an optical density (measured with a Klett colorimeter) corresponding to 4.4×10^8 cells per ml. The cells were centrifuged, suspended in the same volume of 10 mM Tris buffer (pH 8.0), and broken by 2 min of sonication (50% cycle) at 0°C. Large cell debris was removed by centrifugation for 1 min in an Eppendorf (Hamburg, Germany) table centrifuge, and 5-μl samples of the supernatant were analyzed by rocket gel electrophoresis. Control experiments demonstrated that 90% of the capsule polysaccharide originally present on the cell surfaces and free in the growth medium was accounted for in these preparations. Thus, the values given in Tables 1 to 3 in micrograms per milliliter can be considered to represent micrograms of K1 capsular polysaccharide per 4×10^8 cells. We note that under these growth conditions, 10 to 20% of the total capsular polysaccharide was free in the growth medium.

SDS-PAGE. SDS-PAGE was performed using the discontinuous gel system of Laemmli (14) in slab gels with the following modifications. The running gel were 14 cm long, 12 cm wide, and 1.2 mm thick. They contained 4 M urea and an exponential gradient of acrylamide (7.5 to 25.0%, wt/vol) and bisacrylamide (0.2 to 0.67%, wt/vol) generated according to

the formula: $C_i = 7.5 + 17.5 \times e^{-V_i/12.5}$ where C_i is the concentration of acrylamide, and V_i is the volume delivered within the range of 0 and 30 ml. The gradients were generated with an XPO77 gradient maker (Hoefer Scientific Instruments, San Francisco, Calif.) according to the manufacturer's directions. After polymerization, a stacking gel containing 5% acrylamide and 0.13% bisacrylamide was cast. Samples in the sample buffer of Lugtenberg et al. (15) of 3 to 5 μl were loaded, and electrophoresis was performed in the gel buffer of Lugtenberg et al. (15), but containing 0.2% SDS, at 10 mA per gel for 16 h. The resolution obtained was dependent on using these conditions and was strongly disturbed by minor modifications. Silver nitrate staining was performed with periodic acid treatment by the method of Tsai and Frasch (31). In our hands, this method stains LPS more efficiently than proteins, and the major bands seen in cell membrane preparations correspond to LPS. For protein staining, the gels were incubated at 60°C for 60 min in 0.2% Coomassie brilliant blue in 50% (vol/vol) methanol–10% (vol/vol) acetic acid. They were then destained by two incubations for 60 min each at 60°C in 10% acetic acid. Autoradiography was performed at room temperature for periods between 2 days and 3 weeks with dried gels and X-Omat R film (Eastman Kodak Co., Rochester, N.Y.).

Rocket gel electrophoresis. Immunoglobulins were precipitated from horse 46 anti-*N. meningitidis* group B serum with 40% ammonium sulfate at 4°C overnight, followed by extensive dialysis against 100 mM Tris–200 mM NaCl (pH 8.0). The volume was then reduced fourfold by dialysis against 30% polyethylene glycol, and the antibodies were stored at −30°C. The gels used for electrophoresis contained 1% (wt/vol) agarose, 0.5% polyethylene glycol 6000, and 0.25% of the antibody preparation in barbital buffer. A 12-ml sample of the antibody-agarose solution was poured on each 9.5- by 8.5-cm (precoated) glass plate, 17 2.5-mm (diameter) holes were punched, and 5-μl samples were loaded. Electrophoresis was conducted at 2 V/cm for 15 h at 10°C. The gels were stained with Coomassie brilliant blue (33). When purified K1 capsule polysaccharide was used to generate a standard curve, 0.02 μg/ml yielded a detectable reaction. The method was useable to measure K1 capsule concentrations in the range from 0.2 to 25 μg/ml by reading from the standard curve. In most cases, the concentrations presented here represent the arithmetic mean values (±1 standard deviation) obtained with two independent capsule preparations. Values listed without indication of the standard deviation represent those cases where only one preparation was analyzed. This method was not suitable for assaying O-acetyl$^+$ preparations because these polysaccharide yielded only a very diffuse reaction with the antiserum used.

P and type I fimbriae. P fimbriae and X adhesins were tested using CFA agar grown bacteria with human P^1 and p erythrocytes as described previously (13, 32). Mannose-sensitive agglutination of yeast cells by bacteria grown in static L broth, performed as described previously (12), was taken to demonstrate the presence of type I fimbriae. In some cases, repeated determinations, yielding the same results, were performed on different occasions.

RESULTS

Fimbriae among clonal representatives. The clonal representatives were tested by specific agglutination tests for production of fimbriae (Table 2). O1:K1 strains of membrane protein pattern 5 were heterogeneous; most had neither type I nor P fimbriae, whereas a few made either type I fimbriae or both type I and P fimbriae. The other clonal groups were each homogeneous, with only a few exceptions. Type I fimbriae were found on almost all strains. P fimbriae were found within five clonal groups, but were lacking on strains of the two O18:K1 and the O75:K100 groups.

Capsule production by the K1 clonal groups. Ouchterlony precipitation tests did not reveal any differences in the antigenic specificity of K1 polysaccharide of the different clonal groups (data not shown). A sensitive rocket gel electrophoresis method was developed to assay K1 capsule production. Only minor differences were observed in each group in the amount of K1 capsule produced by most of the strains; 12 of 80 of the K1 strains synthesized much more or much less K1 capsule than did the other members of the corresponding clonal group. These may represent mutations that have accumulated during storage in the laboratory, and these 12 strains were excluded from the calculation of the average capsule production (Table 2).

Although most of the clonal groups tested synthesized

TABLE 2. Uniformity of LPS, K1 capsule, and fimbriation among members of the clonal groupings[a]

Serotype	Membrane protein pattern	No. of strains tested	Avg capsule produced[b]	LPS type[c]	No. with fimbriae			
					P,I	P	I	Neither
O1:K1[d]	5	16	23.0 ± 0.9	O1:K1/5	3	0	3	10
O1:K1[e]	9	17	18.0 ± 1.3	O1:K1/9	15	1	1	0
O7:K1[f]	3	13	17.2 ± 1.3	O7:K1	12	0	1	0
O16:K1[g]	12	13	17.8 ± 1.6	O16:K1	10	2	0	0
O18:K1[h]	6	10	16.2 ± 1.2	O18:K1	0	0	10	0
O18:K1[i]	9	11	16.6 ± 0.8	O18:K1	0	0	11	0
O18:K5	11	11	NA[j]	O18:K1	10	1	0	0
O18:K5	18	7	NA	O18:K5/18	7	0	0	0
O75:K100	11	7	NA	O75:K100	0	0	6	1

[a] The results presented here are a summary of the individual results listed in Table 1.
[b] The averages were calculated excluding the obvious exceptions which are listed in footnotes *d* to *i*.
[c] The convention defined in footnote *c* of Table 1 is used here. Exceptional strains are listed in footnotes *d* to *i*.
[d] Exceptional strains (capsule: 3, 25) were ignored.
[e] Exceptional strains (capsule: 29; LPS: 28 to 30) were ignored.
[f] Exceptional strains (capsule: 174, 185, 189; LPS: 174) were ignored.
[g] Exceptional strains (capsule: 204, 207, 211; LPS: 202) were ignored.
[h] Exceptional strains (capsule: 108, 113; LPS: 113) were ignored.
[i] Exceptional strains (capsule: 78) were ignored.
[j] NA, Not applicable because these strains do not make K1 capsule.

comparable amounts of K1 capsule, members of the O1:K1 protein pattern 5 group synthesized significantly more capsule than did members of the other groups ($P < 0.001$, double-tailed t test to compare means). Thus a representative of this clonal group should result in high yields when used for K1 capsule purification. We note that strain number 3 (original designation K235) produced exceptionally little K1 capsule (8.3 μg/ml; Table 1). This strain has commonly been used for the purification of K1 polysaccharide and has also served as the reference colicin K-producing strain. Two isolates, used independently as reference strains for these two properties, and which have been maintained separately for 2 decades, were both tested. Both produced colicin K and comparably low amounts of K1 capsule polysaccharide.

Analysis of LPS by SDS-PAGE. LPS from smooth *E. coli* strains can be resolved by SDS-PAGE into numerous molecular species of differing oligosaccharide chain length (6, 20). These species can be detected by autoradiography with radioactive preparations (6) or by staining with silver nitrate (31).

^{33}P-labeled cell envelopes were analyzed by autoradiography. The bands detected (Fig. 1) could be assigned to different molecular species: phospholipids, a phosphoprotein (see below), lipid A core, and smooth LPS containing between 1 and 47 repeating oligosaccharide units. In agreement with previous reports (6, 20), the smooth LPS bands were present at differing concentrations such that three clusters (labeled Pos. I, II, and III in Fig. 1) were predominant. The phospholipid band was identified on the basis that it comigrated with purified, ^{33}P-labeled phospholipids.

Silver nitrate stained one protein band intensely in addition to the lipid A core and smooth LPS molecules (Fig. 2). However, the resolution with this method was poor when cell envelopes were analyzed and improved when Sarkosyl-insoluble preparations were tested. Although half of the LPS molecules in the outer membranes are removed by such detergent treatment, the size distribution of the remainder was indistinguishable from that found with untreated membranes. LPS preparations purified from O1, O7, or O18 bacteria by the hot phenol-water method (34) contained the same bands (other than the protein band) seen with cell membrane preparations. We note that less lipid A core material was detected in these purified preparations than in the cell membranes. Cell membranes from the rough laboratory strain, *E. coli* K12, contained lipid A core, but no smooth LPS molecules, as expected.

The phosphoprotein band detected by autoradiography was present at comparable intensity whether cell membranes containing predominantly smooth or predominantly rough LPS molecules were analyzed and is particularly visible in those tracks lacking smooth LPS (Fig. 3). This band was also detectable with preparations from capsule-deficient mutants and was not detected by silver nitrate staining. However, no direct evidence is yet available that this band does indeed represent a protein. The phospholipid band was present at comparable concentration in all of the samples analyzed.

Standard patterns of the clonal groups. A total of 105 members of the 9 clonal groups (2) were tested. Each clonal group was highly uniform in regard to both the length distribution and the migration pattern of the LPS (Tables 1 and 2). The individual migration patterns of the clonal groups

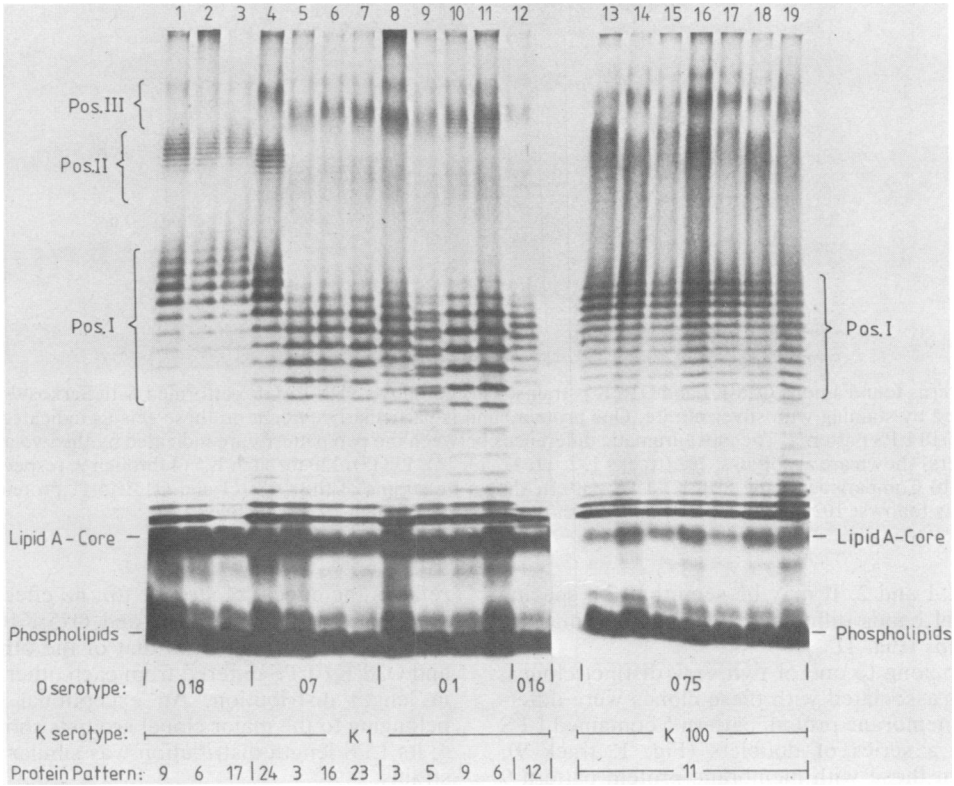

FIG. 1. LPS patterns characteristic of the clonal groups. ^{33}P-labeled membranes were analyzed by autoradiography. The strains shown (track number) are: 64 (1), 102 (2), 118 (3), 191 (4), 165 (5), 190 (6), 180 (7), 22 (8), 9 (9), 41 (10), 58 (11), 205 (12), 215 through 221 (13 through 19, respectively).

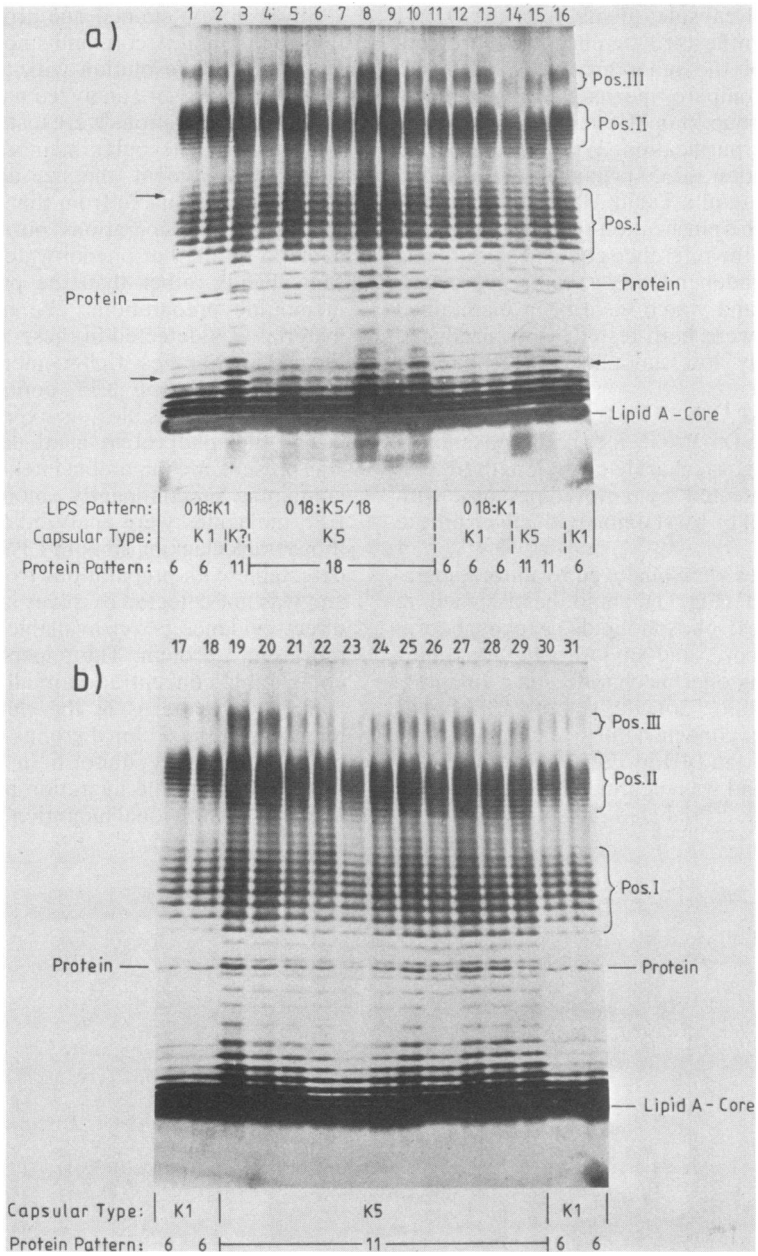

FIG. 2. LPS patterns found among O18-K1 and O18:K5 strains. This gel shows SDS-PAGE performed with Sarkosyl-insoluble membrane preparations followed by staining with silver nitrate. One protein band is particularly evident on these gels as indicated. (a) Comparison of O18:K1 and O18:K5/18 LPS patterns. The most dramatic differences between the two patterns are indicated by the two arrows. The bacterial strains (track numbers) shown are as follows: 102 (tracks 1, 2, 11, 12, 13, 16); 138 (3); 120 through 125 (4 through 9, respectively); 137 (10); 126 (14); and 127 (15). (b) Comparison of the O18:K1 LPS pattern shown by strains of the O18:K1 and O18:K5/11 protein types. The strains (tracks) shown are as follows: 102 (17, 18, 30, 31) and 126 through 136 (19 through 29, respectively).

are shown in Fig. 1 and 2. It may be seen that the spacing between individual bands differed between representatives of the clonal groups (Fig. 1).

O1:K1 strains belong to one of two very distinct clones. The LPS patterns associated with these clones were different. Strains with membrane protein pattern 5 contained LPS that migrated as a series of doublets (Fig. 1, track 9), whereas LPS from those with membrane protein pattern 9 migrated as a series of single bands, each comigrating with the uppermost band of the doublet pattern (Fig. 1, track 10). It has been proposed that the existence of double bands reflects the effects of storage (6); no effects of storage were seen here. LPS of O16:K1 and O75:K100 strains differed from each other and from that of the other groups. O7:K1 and O18:K1 LPS differed from each other most dramatically in length distribution. An exceptional O7:K1 strain not belonging to the major clonal group is shown in Fig. 1, track 4; its LPS length distribution was similar to that of O18:K1 strains.

Two O18:K1 (sub)clones and two O18:K5 (sub)clones were recognized formerly (2). LPS of the two O18:K1 subclones was indistinguishable by SDS-PAGE (Fig. 1,

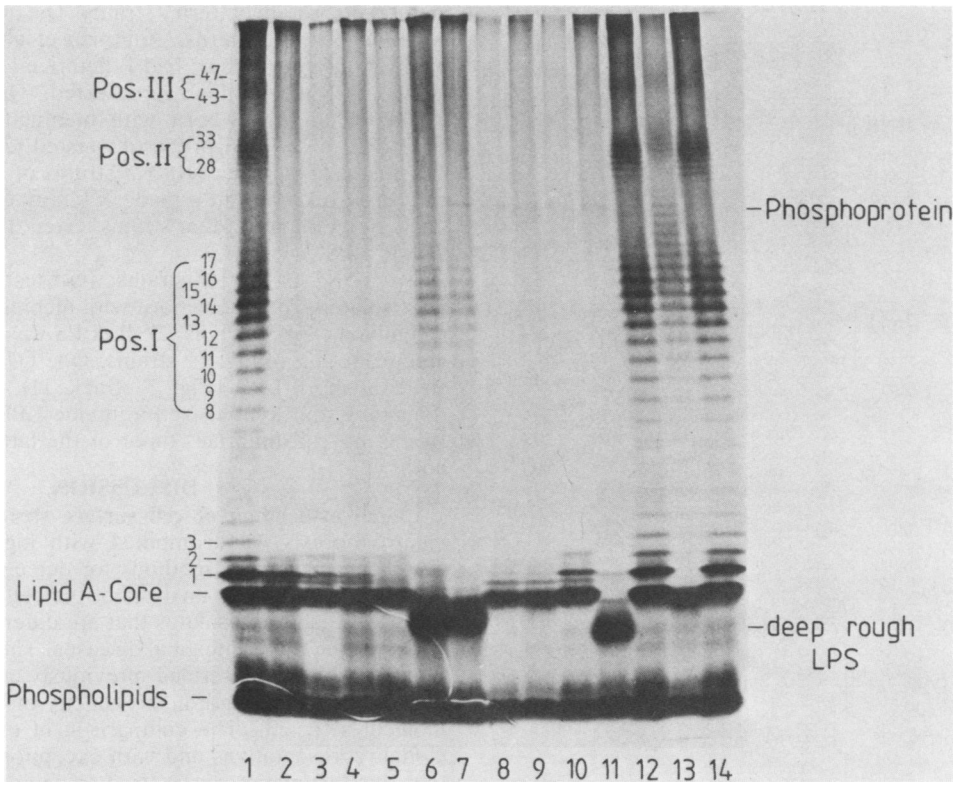

FIG. 3. SDS-PAGE of mutant strains with altered length of LPS or with altered lipid A-core. ^{33}P-labeled membranes were analyzed by autoradiography. Tracks 1, 12, and 14 contain LPS from an O18:K1 strain, no. 102, whereas track 13 contains LPS from strain no. 117. The other strain numbers are given in Table 3. The sequential numbers from 1 to 47 indicate the number of oligosaccharide repeat units. These numbers were determined using other exposure times and including the data from other gels containing LPS with bands migrating at positions between those labeled I, II, and III.

tracks 1 and 2) and manifested the same interband spacing as that of the O18:K5 strains of protein pattern 11 (Fig. 2b). LPS from these O18:K5 strains contained increased amounts of the bands between lipid A core and position I as compared to the LPS from the O18:K1 cells. Despite these differences, LPS from all three groups is referred to as O18:K1 LPS in Tables 1 and 2. The other O18:K5 subclone (membrane protein pattern 18) synthesized an LPS that was subtly different in interband spacing from that of the three other clonal groupings (Fig. 2a; this difference is most dramatic at the positions marked by arrows). This LPS is referred to as O18:K5/18 LPS in the tables. All of these strains are classified as O18ac. O18ab strains were different and will be described elsewhere (M. Achtman, A. Moll, B. Kusecek, G. Pluschke, B. Slawig, B. Jann, and K. Jann, manuscript in preparation).

LPS variants among clonal members. One real exception to these generalizations was found, namely, an O16:K1 strain (no. 202), which showed a different migration pattern from that typical of O16:K1 strains and which will be described elsewhere (Achtman et al., in preparation). Two strains were found (113, Fig. 3, track 7; 29, Fig. 3, track 10) which appear to represent deep rough and O^- mutants, respectively (see below). Three minor variants made a shorter LPS (Fig. 4, track 3) or fewer long LPS chains (Fig. 4, tracks 6 and 8).

Analysis of mutant strains. Mutant strains were analyzed to substantiate the molecular assignments made above and to demonstrate the potential of the SDS-PAGE method. The LPS patterns of strains that synthesized dramatically shortened molecules or molecules with an altered lipid A core (Table 3) are shown in Fig. 3. These strains included mutants isolated in the laboratory after mutagenesis with the transposon Tn*10* or which were selected by treatment with a bacteriophage. The other strains were found during the course of the analyses presented here. Two SpAg:K1 strains isolated from newborns with meningitis (A1317, A1318) (4) were also included. (A third SpAg:K1 strain described in reference 4, no. 182, proved to synthesize so many smooth LPS chains that it should properly not be designated as rough.)

Tn*10*-induced mutants not agglutinated by rabbit anti-O antiserum made considerable amounts of the first smooth LPS band as well as lipid A core and material at a position intermediate between the two (Fig. 3, tracks 2, 3, and 4). The same pattern was found with a spontaneous O^- mutant (Fig. 3, track 10). Another spontaneous O^- mutant (Fig. 3, track 5) and two naturally isolated SpAg:K1 strains (Fig. 3, tracks 8 and 9) synthesized predominantly lipid A core. Three of the mutant strains probably contain deep rough lipid A core because a band migrating considerably more quickly than lipid A core was detected (Fig. 3, tracks 6, 7, and 11). Two of these strains also synthesized considerable quantities of smooth LPS molecules. One smooth strain (Fig. 3, track 13) contained a lipid A core that migrated slightly faster than that of smooth K1 isolates, but contained almost normal amounts of long-chained LPS molecules.

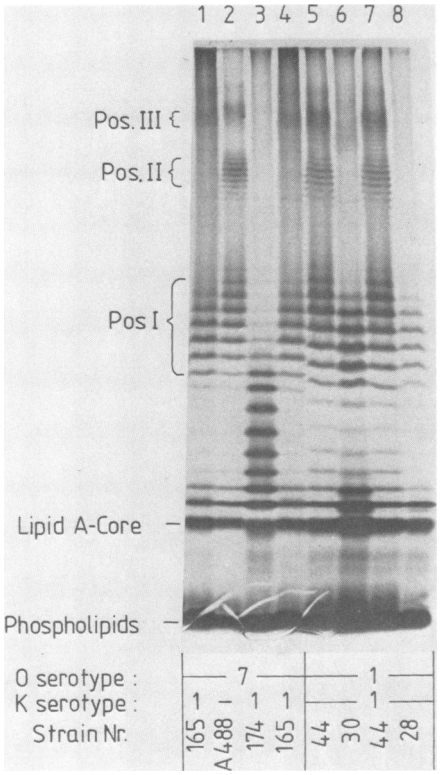

FIG. 4. SDS-PAGE of mutant strains showing slight differences in length of their LPS. ^{33}P-labeled membranes were analyzed by autoradiography.

Upon longer autoradiographic exposures than those shown here, long-chained LPS molecules were detected for all but three of the strains shown in Fig. 3 (tracks 8, 9, and 11). The Tn*10*-induced mutants showed additional bands at intermediate positions between the bands 1 through 6.

Capsule-deficient mutants. Because the mutants A467, A717, and no. 113, which were detected because they were not agglutinated by anti-O antiserum, also synthesized reduced levels of K1 capsule (Table 3), other mutants selected by resistance to capsule-specific bacteriophages were examined in respect to LPS. Mutants synthesizing no detectable capsule were unaltered in the LPS pattern on SDS-PAGE (Table 3), whereas a partially deficient mutant (A488) synthesized more LPS at position II than did its parent strain (Fig. 4, track 2 versus tracks 1 and 4).

K1$^+$ and K5$^+$ strains not belonging to the clonal groupings. Nine strains (3 O1:K1, 4 O7:K1, 1 O18:K1, and 1 O18:K5) not belonging to the clonal groupings on the basis of membrane protein pattern (2) were tested (Table 1). One O1:K1 strain (no. 22; Fig. 1, track 8) and the O18:K5 strain (no. 119) synthesized LPS of unique structure (Achtman et al., in preparation). One O7:K1 strain (no. 191) had an LPS of unusual length distribution (Fig. 1, track 4), made less capsule (7.2 μg/ml) than normal, and did not possess P fimbriae. This strain is the only animal isolate that was tested. The O18:K1 strain (no. 117) made a modified lipid A core (Fig. 3, track 13) and more capsule than normal (23.2 μg/ml). The other five strains (no. 57, 58, 180, 190, and 192) resembled the clonal representatives (Fig. 1, tracks 6, 7, and 11; data not shown).

K? and K$^-$ strains. Four O1:nonK1 strains were tested. One had an LPS indistinguishable from that of O1:K1 membrane protein pattern 9 strains. The other three had LPS of unique band patterns (Achtman et al., in preparation). None of the four strains had P fimbriae (Table 1).

Five O18:K? strains were tested. One (no. 138) was isolated from a newborn with meningitis and resembled O18:K5 strains in all properties tested (2). This strain was indistinguishable from O18:K5 strains of membrane protein pattern 11, except that it made X fimbriae (Table 1; Fig. 2a, track 3). Of the four other strains tested, three synthesized a unique LPS.

Six O7:K$^-$ or O7:K? strains were tested. One (no. 193) was isolated from a newborn with meningitis and otherwise resembled O7:K1 strains (2). Its LPS was also indistinguishable from that of O7:K1 strains. One O7:K$^-$ strain synthesized a short LPS (Fig. 3, track 11), and three of the remaining four synthesized a unique LPS. Whereas O7:K1 strains have P fimbriae, three of the latter five strains did not.

DISCUSSION

Clonal association of cell surface structures. Rocket gel electrophoresis was combined with high-resolution SDS-PAGE and with new methods for detecting P fimbriae (13, 32) for a comparative analysis of the cell surface structures on *E. coli* bacterial isolates that are differentially associated with specific extratintestinal diseases. The SDS-PAGE technique has been described previously as being useful to demonstrate the length heterogeneity of lipopolysaccharide molecules (6, 20). The comparison of mutant strains with clonal representatives and with exceptional isolates reveals that this technique can be used to demonstrate both minor and major differences in chain length as well as chemical differences in LPS structure. Data will be presented elsewhere from adsorption studies and studies with monoclonal antibodies showing that the LPS patterns designated as unique represent molecules that are serologically quite distinct from those synthesized by the clonal representatives, although they have all been assigned to the same O serogroups (Achtman et al., in preparation).

Rocket gel electrophoresis has been used previously to quantitate the amount of K1 polysaccharide made by *E. coli* cells (28). Modifications were introduced to increase the reproducibility and sensitivity of this method such that small but significant differences in the amount of capsular polysaccharide synthesized were detectable.

The bacterial isolates tested were obtained from Europe and the United States over a 40-year period, from healthy and from diseased persons (2). They have been assigned previously to nine clonal groups on the basis of O:K serotype plus outer membrane protein pattern. It is striking that within each clone, with only few exceptions, the bacterial strains were homogeneous for all of the properties tested. Of the 105 bacterial strains from the nine clonal groups (2) tested here, only 6 showed detectable differences in LPS. For five of these six, the LPS molecules were of different length than those representative of the corresponding clones, whereas a compositional difference was detected for the sixth strain. Examination of fimbriation revealed that O1:K1 bacteria of membrane protein pattern 5 were variable in both P and type I fimbriation. Of the remaining 89 strains tested, only 7 differed from the clonal representatives in fimbriation. (An equally striking correlation between some of these serotype groups and fimbrial antigens has been reported by Ørskov and Ørskov [18] for Swedish and Hungarian isolates.) Of 80 K1$^+$ strains (six bacterial clones), 12 differed markedly in the amount of capsular polysaccharide synthesized (Table 3). The rarity of strains variant in the

TABLE 3. Properties of mutant strains

Mutant strain no.	Parent strain[a] (O antigen)	Selection	K1 capsule[b] (% of parent)	LPS	Changes in fimbriae[c]
A463	102 (O18)	LPS::Tn*10*	16.8 (88)	Fig. 3/2	None
A464	102 (O18)	LPS::Tn*10*	17.0 (89)	Fig. 3/3	None
A465	102 (O18)	LPS::Tn*10*	16.8 (88)	Fig. 3/4	None
A467	79 (O18)	Spontaneous	8.5	Fig. 3/5	None
A717[d]	102 (O18)	BP156[r]	8.7 (45)	Fig. 3/6	None
113[a]	(O18)	Spontaneous	10.8	Fig. 3/7	
A1317[e]	(?)	Spontaneous	17.0	Fig. 3/8	
A1318[f]	(?)	Spontaneous	15.8	Fig. 3/9	
29[a]	(O1)	Spontaneous	13.3	Fig. 3/10	
194[a]	(O7)	Spontaneous		Fig. 3/11	
A26[d]	27 (O1)	D[r]	≤0.02 (≤1)	Unchanged	None
A67[d]	101 (O18)	D[r]	≤0.02 (≤1)	Unchanged	X fimbriae
A487[d]	165 (O7)	E[r]	≤0.02 (≤1)	Unchanged	X fimbriae
A488[d]	165 (O7)	E[r]	4.0 (22)	Fig. 4/2	X fimbriae
A691[d]	102 (O18)	BP1[r]	≤0.02 (≤1)	Unchanged	None

[a] These numbers refer to the sequential numbers in reference 2 and which are used in Table 1.
[b] The data are given as micrograms per milliliter (percentage of parental value).
[c] Changes other than in the expression of X fimbriae were not observed. All of the mutant strains expressed X fimbriae. Strains labeled "none" had parents that also expressed X fimbriae.
[d] Isolated as being resistant to the bacteriophages BP156, D, E, or BP1, respectively.
[e] Original designation 412 in reference 4.
[f] Original designation 194 in reference 4.

properties tested here contrasts with the strong variation found between representatives of different clonal groups and found when random isolates were compared. This uniformity substantiates the interpretation (2) that each of the clonal groups represents the linear descendents of one ancestral cell.

Four of the nine K1 or K5 strains tested that differed from the clonal groups in membrane protein pattern also differed in one or more of the properties tested here. The origin of these variant bacteria is not clear; they may represent members of relatively unsuccessful and rarely isolated clones, or they may be distantly related to the common clonal groups. Of the 15 non-K1, non-K5 isolates, 12 differed in one or more of the properties tested from the K1 or K5 bacteria of the corresponding O serotype. Nine of these synthesized LPS that differed in interband spacing, and presumably in chemical structure, from that of the clonal members. These results provide support for the argument (2) that it is inadvisable to group together bacteria sharing any one O antigenic type, but differing in capsular serotype antigen.

It was suggested (2) that most O18:K1 isolates belong to one of two related subclones and that the O18:K5 bacteria belong to two further subclones. The LPS from three of these four bacterial groups were uniform by SDS-PAGE, whereas one of the O18:K5 clones synthesized LPS that differed from that of the other O18 bacteria on SDS-PAGE. The O18:K1 bacteria lacked P fimbriae, whereas the O18:K5 bacteria possessed P fimbriae. Although serological analysis of P fimbriae is complicated by the presence of multiple fimbrial antigens on certain *E. coli* strains (13), we note that an analysis of O18:K5 strains isolated in Sweden and Hungary revealed that all 11 strains tested possessed the F8 fimbrial antigen (18), which may well correspond to the P fimbriae found here on such strains.

It was suggested (2) that O1:K1 bacteria belong to two unrelated clones. The observations presented here support this interpretation. LPS from one group migrated as a series of doublet bands, whereas that from the second group migrated as a series of singlets. Elsewhere we show that the two LPS species possess different antigenic determinants (Achtman et al., in preparation). One of the two O1:K1 groups differed from the other K1 groups tested in that the bacteria synthesized more K1 polysaccharide and that it was not homogenous in regard to fimbriation. Most of the O1:K1 strains of the other clone (membrane pattern 9) possessed P fimbriae and were associated with the H7 flagellar antigen (2). O1:K1:H7 strains isolated in Sweden and Hungary synthesized the F11 fimbrial antigen (18), which may therefore correspond to the P fimbriae detected here.

On further correlation is possible between the P fimbriation seen here and the antigenic assignments of Orskov and Orskov (18), namely, that the O16:K1 strains possess P fimbriae that may correspond to the F12 antigen. Such a correlation is not possible for the O7:K1 strains because some of the strains tested by Orskov and Orskov (18) possessed a different flagellar antigen from that typical of the clonal group tested here.

Relationship of cell surface structures to virulence. The clonal groups are differentially associated with specific disease processes as follows (Table 4). O1:K1 and O18:K5 bacteria are commonly isolated from feces and from patients with urinary tract infection, including pyelonephritis (9, 19, 23, 24). These bacteria are rarely isolated from newborns with meningitis (23) and are inefficient at causing bacteremia after colonization of newborn rats (22). These bacteria are also sensitive to the bactericidal effects of the serum complement system even in the absence of specific antibodies (21). O18:K1 bacteria are common causes of newborn meningitis (24), are invasive in the neonatal rat model (22), and are resistant to complement killing unless anti-LPS or anticapsule antibodies are added (21). In contrast, O18:K1 bacteria are rarely isolated from cases of urinary tract infection (22). In fact, recent investigations indicate that, although they may cause bladder infections, they are very rare causes of pyelonephritis (19; Korhonen, unpublished data; personal communication from S. Marild, Department of Clinical Immunology, University of Goteborg, Sweden). Finally, O7:K1 bacteria are associated with newborn meningitis, pyelonephritis, and invasion of newborn rats and are resis-

TABLE 4. Virulence factors implicated in diseases associated with certain groups of K1 and K5 *E. coli*

Disease(s)	Virulence factor(s)	Groups possessing virulence factor	Group lacking virulence factor
Newborn meningitis and invasion of rats	K1 capsule	O18:K1; O7:K1	O18:K5
	LPS type	O18:K1; O7:K1	O1:K1
Pyelonephritis	P fimbriae	O1:K1 O18:K5 O7:K1	O18:K1

tant to complement killing unless antibodies are added (21).

Recent results (32) have implicated P fimbriae as critical virulence factors for bacteria causing pyelonephritis. In agreement, those bacteria associated with pyelonephritis (O1:K1, O7:K1, O18:K5) possessed P fimbriae whether isolated from diseased or healthy persons, whereas those not associated with pyelonephritis (O18:K1 and O75:K100) did not. It seems, however, that possession of P fimbriae is irrelevant to newborn meningitis and invasion of neonatal rats because of O18:K1 bacteria are P^-, whereas O7:K1 bacteria are P^+, although they are equally invasive.

The disease specificity seen for newborn meningitis, invasion of neonatal rats, and antibody-independent activation of the complement system could not be accounted for by any detectable diffences in the amount of capsular polysaccharide synthesized or the amount or the average chain length of LPS. The noninvasive O1:K1 bacteria synthesized as much, or more, K1 capsular polysaccharide as did O7:K1 or O18:K1 bacteria. No immunological differences were detected among capsule preparations from the different clonal groupings by using Ouchterlony tests (data not shown). The length distribution of O1 LPS was the same as that of O7 LPS, and the amounts of LPS detected in membrane preparations were comparable. Thus, the amount and length of LPS and the amount of capsule made cannot account for these virulence specificities. However, these results do not indicate that either capsule or LPS is irrelevant to the infectious process. O^- and K^- mutants are noninvasive in neonatal rats (21), and epidemiological data implicate the K1 capsule as a virulence determinant for newborn meningitis (23). The chemical composition of both the capsule and the O antigen determine the sensitivity of the bacteria to antibody-independent activation of the serum complement system (21). The K1 capsule impedes activation of the alternative complement pathway, whereas certain O antigens can protect against antibody-independent activation of the classical pathway (21). Furthermore, purified O1 LPS activates the classical complement pathway directly (Pluschke and Achtman, submitted). The only differences detected, using a large variety of tests (2; Mercer et al., in preparation), between O1:K1 and O18:K1 bacteria of membrane protein pattern 9 reside in their LPS and P fimbriation. P fimbriae seem to be irrelevant to invasion and resistance to the complement killing (see above). Thus it seems that the chemical structure of the LPS accounts for the differences in invasion that distinguish these bacteria (Table 4).

The proposal that specific smooth LPS types are virulence determinants poses an apparent paradox because approximately 20% of K1 *E. coli* isolates from newborn meningitis are spontaneously agglutinating in saline (4, 24). Two of three such strains tested here were shown to synthesize a rough LPS consisting predominantly of lipid A core. However, apparently many (other) SpAg:K1 isolates retain sufficient smooth LPS molecules that they can still be O typed (P. Gemski, personal communication) and should therefore not be classified together with rough strains. Furthermore, it is unclear whether the two SpAg:K1 strains tested here were rough before isolation or whether they might represent mutations that accumulated during laboratory passage. Such mutations in other strains have been documented here. All rough mutants tested, including A1317 and A1318, were noninvasive in the neonatal rat model. However, it remains possible that patients from whom SpAg:K1 strains are isolated lack defense mechanisms that normally kill O^- strains. The results from bacteremia in rats do not apply to bacteremia in adults; O1:K1 bacteria are commonly isolated from adult bacteremia. However, it remains possible that some bacteremic adults also lack defense mechanisms that would otherwise kill O1:K1 or rough strains.

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