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Virulence Patterns and Long-Range Genetic Mapping of Extraintestinal *Escherichia coli* K1, K5, and K100 Isolates: Use of Pulsed-Field Gel Electrophoresis

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A total of 127 extraintestinal *Escherichia coli* strains of the capsule serotypes K1, K5, and K100 from human and animal sources were analyzed for DNA sequences specific for the genes for various adhesins (P fimbriae [*pap*] and P-related sequences [*prs*], S fimbriae [*sfa*]/F1C fimbriae [*foc*], and type I fimbriae [*fim*]), aerobactin (*aer*), and hemolysin (*hly*). The expression of corresponding virulence factors was also tested. Twenty-four selected strains were analyzed by long-range DNA mapping to evaluate their genetic relationships. DNA sequences for the adhesins were often found in strains not expressing them, while strains with hemolysin and aerobactin genes usually did express them. Different isolates of the same serotype often expressed different virulence patterns. The use of virulence-associated gene probes for Southern hybridization with genomic DNA fragments separated by pulsed-field gel electrophoresis revealed that a highly heterogeneous restriction fragment length and hybridization pattern existed even within strains of the same serotype. Long-range DNA mapping is therefore useful for the evaluation of genetic relatedness among individual isolates and facilitates the performance of precise molecular epidemiology.

Escherichia coli strains of the K1 capsular serotype represent 80% of all *E. coli* strains isolated from cases of newborn meningitis and sepsis in humans (24, 57, 63). Moreover, K1 strains and isolates of other serotypes, including O18:K5 and O75:K100, are able to cause urinary tract infection, and K1 *E. coli* isolates are also often found to be the causative agent of systemic infections in animals (3, 48, 64, 70, 71).

It has been clear for several years that the capsular antigens, especially K1 and certain types of O antigen, are strongly associated with such extraintestinal infections (4, 33, 60, 61, 64), as are specific fimbrial adhesins (18, 42, 45, 46), hemolysin (Hly) (20, 22, 23), and aerobactin (Aer) (11, 28, 37). Extraintestinal *E. coli* isolates may carry different types of fimbrial adhesins which can be distinguished serologically (47) and can show different binding properties. P fimbriae (also termed Pap pili [45]) are associated with pyelonephritis and recognize an α-D-galactosyl-(1-4)-β-galactose receptor (29, 66). P-related sequences (Prs) mediate binding to galactosyl-*N*-acetyl-(α1-3)-galactosyl-*N*-acetyl residues (40). The S-fimbrial adhesin (Sfa) interacts with α-sialyl(2-3β)-D-galactose receptor molecules (32, 54). Another adhesin, the type I fimbrial adhesin, is produced by pathogenic and nonpathogenic strains and is able to bind to α-D-mannose-containing receptors (47). Fimbriae of serotype F1C are unable to agglutinate erythrocytes but do seem to interact with cells of the human urinary tract (56, 68, 69).

E. coli strains may be subdivided into clones by electrophoretic typing of alloenzymes (4) and by outer membrane protein (OMP) profiles (3). In some cases, the clones have a characteristic serotype based on the O lipopolysaccharide and K capsular antigens (4). Attempts have been made to correlate the expression of the various virulence factors to the clonal types. In such studies, however, the phenotypic status of expression was tested rather than the presence of the virulence genes on the genome. Recently, DNA probes specific for sequences coding for hemolysin (20, 25, 44), aerobactin (11, 28, 35, 37, 49, 67, 71), capsule antigens (58), and various adhesins, such as Pap (6, 13, 38, 39), Sfa (21, 22, 52), and type I fimbriae (30), have been developed to enable genetic mapping.

In this publication, we report on the distribution and the phenotypic expression of five different virulence factors within *E. coli* isolates belonging to different clonal groups with the capsule antigens K1, K5, and K100. Furthermore, the newly developed technique of pulsed-field electrophoresis of genomic DNA (36) was applied to selected strains to gain information on the location of virulence genes on the genome and on the genetic relatedness of strains. These data are useful for studies on molecular epidemiology.

MATERIALS AND METHODS

Chemicals, medium, and enzymes. Bacteria were grown in L-broth as described before (21). Strains carrying recombinant plasmids were cultivated under selective antibiotic pressure with ampicillin (50 μg/ml), tetracycline (20 μg/ml), and chloramphenicol (15 μg/ml). Antibiotics were from Bayer, Leverkusen, Germany; restriction enzymes were from Boehringer, Mannheim, Germany; and all other chemicals were from Sigma, Deisenhofen, Germany.

Bacterial strains. A total of 127 wild-type *E. coli* strains were tested (Table 1). Of these, 109 isolates carry the K1 capsule, 14 strains belong to serotype O18:K5, and four strains belong to serotype O75:K100. Most isolates were from the Max-Planck-Institut für Molekulare Genetik, Berlin, Germany, including strains of serotypes O2:K1 and

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TABLE 1. Incidence of virulence factors among *E. coli* strains

O:K/OMP	No. of strains tested	% of strains with virulence factor										
		P	Prs	*pap*/*prs*	S	*sfa*	Fim	*fim*	Aer	*aer*	Hly	*hly*
O1:K1/5	6	17	—[a]	83	—	—	50	100	—	83	—	—
O1:K1/9	5	100	20	100	—	—	20	100	80	100	—	—
O2:K1/6	4	100	—	100	—	—	75	100	25	25	—	—
O2:K1/9	16	—	—	25	25	25	63	100	75	75	75	75
O2:K1/29	3	—	67	67	100	100	100	100	33	3	100	100
O2:K1/30	2	—	—	—	—	100	100	100	100	100	—	—
O2:K1/31	2	100	—	100	—	—	50	100	100	100	—	—
O7:K1/3	8	100	—	100	—	—	63	100	100	100	—	—
O16:K1/12	8	88	50	100	—	—	13	100	100	100	63	63
O18:K1/6	7	14	57	100	86	100	57	100	43	43	86	100
O18:K1/9	12	—	—	42	67	84	75	100	42	50	34	42
O45:K1/9	26	43	15	50	—	—	93	100	81	85	—	—
O83:K1/32	10	—	—	—	20	100	50	100	100	100	—	—
O18:K5/18	5	100	100	100	—	—	80	100	100	100	100	100
O18:K5/11	9	100	77	100	—	100	—	100	100	89	100	89
O75:K100/11	4	—	—	—	—	—	50	100	—	100	—	—
Total	127	41	20	61	20	37	61	100	72	80	35	35

[a] —, not detected.

O83:K1 (2–4, 34, 50). O45:K1 strains were from the collection of the Institut für Experimentelle Epidemiologie, Wernigerode, Germany (65, 71). They were assigned OMP designations in accordance with earlier results (3). The other strains were obtained from the collections of the Walter Reed Army Institute, Washington, D.C. (12); the Department of General Microbiology, University of Helsinki, Helsinki, Finland; the Institut für Medizinische Mikrobiologie, University of Lübeck, Lübeck, Germany; the Institut für Experimentelle Medizin und Biologie, Borstel, Germany (19, 50); and the Institut für Genetik und Mikrobiologie, University of Würzburg, Würzburg, Germany (31, 50). The serotypes and the outer membrane patterns of most strains have already been described, and they were determined only for the new strains. The bacteria were characterized by conventional microbiological techniques. *E. coli* K-12 strain HB101 was used as a carrier for recombinant DNA (9).

Determination of adhesins. The presence of mannose-resistant adhesins was determined by hemagglutination assays with human, sheep, and bovine erythrocyte suspensions obtained locally with and without 2% mannose as described before (21, 47). For this purpose, strains were grown on solid LB or CFA medium. P-specific adherence was detected by agglutination with P1 human blood cells and galactose-coated latex beads obtained from Orion (Espoo, Finland) (29, 40). Prs-specific adhesins were determined via agglutination with sheep erythrocytes (40). S-specific binding was assayed in a hemagglutination test with bovine erythrocytes incubated with neuraminidase or fetuin as described before (21, 32, 53). Type I adhesins were detected by mannose-sensitive adherence of strains to *Saccharomyces cerevisiae* cells following cultivation of isolates in liquid medium (47).

Aerobactin bioassay. The aerobactin cross-feeding bioassay was modified from that of Braun et al. (10) as follows. Supernatants of the K1, K5, and K100 strains were applied on filter disks and incubated overnight on plates containing 10^9 cells of the aerobactin-requiring indicator strain *E. coli* K-12 EN99 and a 200 mM concentration of the iron chelator substance 2′,2′-dipyridyl (Sigma) in M9 soft agar. Aerobactin production by the test strains resulted in a zone of enhanced growth of EN99 around the disks.

Hemolysin production. Hemolytic activity was detected by cultivation of strains on blood agar plates and confirmed by a liquid test (20, 23).

DNA techniques. Plasmid DNA isolation, DNA cleavage with restriction enzymes, agarose gel electrophoresis, and elution of DNA fragments from agarose gels were performed as described by Maniatis et al. (41).

Generation of DNA probes. The DNA probes specific for P fimbriae (*pap*), S fimbriae (*sfa*), type I fimbriae (*fim*), aerobactin (*aer*), and hemolysin (*hly*) are shown in Fig. 1. As the *pap*-specific probe, a *Hin*dIII fragment isolated from the plasmid pRHU845 (Tc^r) was used (45). This probe is also specific for the *prs* gene cluster coding for P-related adhesins (40). As the *sfa*-specific DNA, a 1.8-kb *Cla*I-*Eco*RI fragment of pANN801-13 (Ap^r) was used (21, 59). This fragment was also subcloned into pBR322, resulting in the plasmid pANN801-21 (Ap^r). This probe also detects *foc*-specific sequences, which code for F1C fimbriae (51, 52). The 6.0-kb *Pst*I fragment of plasmid pPKL4 (Ap^r) was used as a probe for *fim* DNA (30). For *aer*, a 7.0-kb *Hin*dIII-*Eco*RI fragment of plasmid pRG12 (Ap^r) was used (16). The 3.2-kb *Hin*dIII fragment of plasmid pANN215 (Cm^r) was used as an *hly*-specific probe. It originated from the Hly plasmid pHly152 (44).

DNA dot blot procedure. DNA probes were ^{32}P-labeled by using the random priming system from Boehringer. The DNAs were denatured by heating for 10 min at 95°C and subsequently cooled on ice. The DNAs were added to a mixture of dATP, dGTP, dTTP, and hexanucleotides in 10× concentrated reaction buffer. Then 5 μl of [^{32}P]CTP (3,000 Ci/mmol) and 1 μl of Klenow enzyme were added up to a final volume of 20 μl in an aqueous solution. Incubation time was 30 min at 37°C. The reaction was stopped by heating for 10 min at 65°C, and the reaction mix was cooled on ice for 5 min before the probe for hybridization was used (14). Colony dot hybridization was performed as described previously (41). Stringent conditions were used for washing: one wash for 30 min at room temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0])–0.1% sodium dodecyl sulfate (SDS) and then four washes for 30 min each at 56°C in 0.1× SSC–0.1% SDS.

OFAGE. For orthogonal field alternation gel electropho-

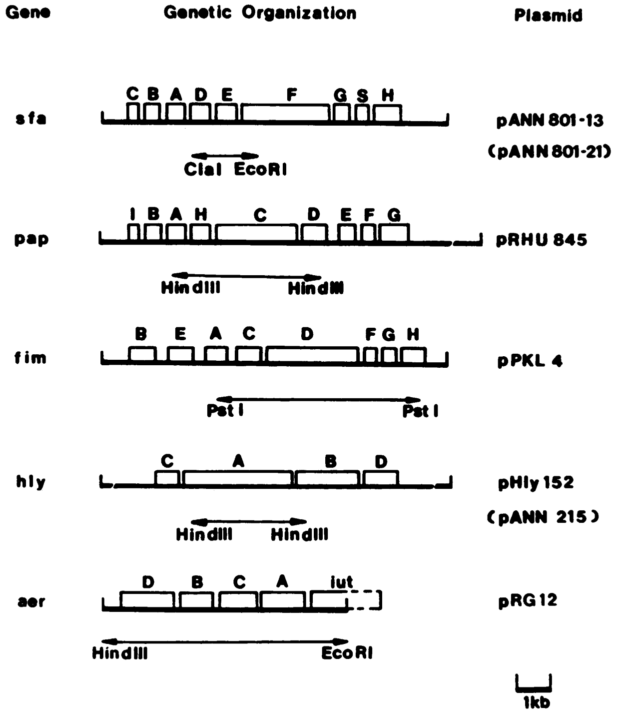

FIG. 1. Gene probes used in this study. The DNA fragments used as gene probes are indicated by horizontal arrows at restriction enzyme recognition sites. The gene clusters specific for *sfa*, P fimbriae (*pap*), *fim*, *hly*, and aerobactin (*aer*) are shown. The boxes designated by capital letters are the cistron loci identified for these determinants. Plasmid designations are on the right.

resis (OFAGE), genomic DNA used for long-range DNA mapping was isolated as described recently (7, 17). Agarose blocks were equilibrated in restriction enzyme buffer for 3 h on ice. Cleavage with *Xba*I restriction enzyme was performed in fresh buffer according to the manufacturer's instructions (Boehringer). OFAGE was performed with a Consort device (Consort, Turnhout, Belgium). The electric fields, arranged at a 120° angle, alternated every 20 s at a constant voltage of 250 V. Electrophoresis was carried out for 72 h at 14°C in 1% agarose gels with 0.5× TBE (41) as the running buffer. Lambda concatemers (Pharmacia, Freiburg, Germany), yeast chromosomes (*S. cerevisiae* WAY 5-4A; Biometra, Göttingen, Germany), and *Hin*dIII-cleaved lambda DNA were used as size markers.

Southern hybridization. Cleaved genomic DNA, separated in agarose gels (see above), was transferred to nitrocellulose paper as described before (62). Radiolabeling, hybridization, and washing were performed as above. For rehybridization, Southern blot filters were incubated for 10 min in 10 mM EDTA (pH 7.5) at 95°C. Complete removal of the DNA probe was confirmed by autoradiography for at least 3 days.

RESULTS

Presence and phenotypic expression of virulence factors. A total of 127 *E. coli* isolates formerly assigned to different clonal groups were analyzed by DNA dot blots for the presence of different virulence-associated genes (Fig. 1). The phenotypic expression of these genes was also tested. The results are summarized in Table 1.

P and Prs fimbrial adhesins. A total of 41% of the isolates produced adhesins which enabled binding to Gal-Gal receptor molecules (P-specific binding), while 61% hybridized with a *pap* gene probe. This gene probe has homology with the P-related sequences (*prs* [40]). Prs adhesins, which mediate agglutination of sheep erythrocytes, were expressed in 20% of the strains, in some cases along with P fimbriae. P-fimbrial adhesins are well expressed by strains of serotypes O1:K1/OMP9 (100%), O7:K1/OMP3 (100%), O16:K1/OMP12 (88%), O45:K1/OMP9 (43%), and OMP18 or OMP11 O18:K5 (100%). The Prs phenotype was detected with a high incidence in O18:K5 strains (89%), as well as in strains of serotypes O2:K1/OMP29 (67%), O18:K1/OMP6 (57%), and O16:K1 (50%). The *pap*/*prs*-specific gene cluster was also detected on 44% of O2:K1 and 63% of O18:K1 isolates of different OMP types, but such strains seldom mediate P-specific binding; rather, Prs-specific agglutination was found in O2:K1/OMP29 and O18:K1/OMP6 strains.

S-fimbrial adhesins. Of the strains, 37% were *sfa* positive in DNA-DNA dot blots, but only 20% of the isolates expressed S-specific binding properties. The S-specific gene probe also detects F1C coding sequences (*foc*) (51, 52). This seems to be the case for the O18:K5 isolates of OMP11, which are known to be F1C positive (50, 56). S-specific binding was found for isolates of different OMP types belonging to serotypes O2:K1 (26%), O18:K1 (74%), and O83:K1 (20%). The presence of *sfa* coding sequences was found exclusively among these serotypes, and only in the case of O83:K1 isolates was there a marked discrepancy between genotype and phenotype.

Type I fimbriae. In our collection, all strains contained *fim*-specific sequences, but only 61% expressed type I fimbriae.

Aerobactin. Aerobactin production was detected for 72% of the isolates, whereas 80% of the strains carry the *aer* gene cluster. Isolates of serotypes O1:K1/OMP5 and O75:K100 were especially likely not to express the *aer* genes. One strain of serotype O18:K5/OMP11 was positive in the bioassay but gave no hybridization signal.

Hemolysin. Thirty-five percent of the strains were hemolytic and generally carried the corresponding genes. Two strains of serotype O18:K1, however, were nonhemolytic but hybridized with the *hly*-specific gene probe, and one O18:K5/OMP11 strain was hemolytic but gave no hybridization signal. Hemolysin is preferentially expressed by strains of serogroups O2:K1/OMP9 and -OMP29 and O16:K1/OMP6 and both K1 and K5 O18 strains.

Combination of virulence factors. The five virulence-associated gene clusters were only detected in combination on the genomes of O18:K5/OMP11 isolates. None of the strains, however, expressed all the factors, because these strains did not express type I fimbria-specific binding; furthermore, they produced F1C fimbriae instead of S-fimbrial adhesins. It is interesting that strains producing S fimbriae seldom expressed P fimbriae; rather they expressed Prs-specific binding (strains of serotypes O2:K1/OMP29 and O18:K1/OMP6). In addition, the coexpression of hemolysin and aerobactin was observed for a few strains (serotypes O18:K5, O2:K1/OMP9 and -OMP29, O16:K1, and O18:K1). Some strains expressed only one virulence factor. The data in Table 1 show no obvious correlation of the virulence patterns with serotype except for a few strains of serotypes O18:K5 and O75:K100, which displayed similar genotypes and phenotypes.

Restriction fragment length polymorphism. Twenty-four representative strains were selected for further analysis by long-range DNA mapping. The virulence patterns of these

TABLE 2. Characterization of *E. coli* strains

No.	Strain	O:K:H/OMP	Adhesins							Aerobactin		Hemolysin		Isolate source[a]	Reference(s)
			P	Prs	*pap/prs*	Sfa	*sfa/foc*	Fim	*fim*	Aer	*aer*	Hly	*hly*		
1	A202	O1:K1:H?/5	−	−	+	−	−	+	+	−	+	−	−	Human feces	3, 34
2	A200	O1:K1:H?/5	−	−	−	−	−	−	+	−	+	−	−	Human feces	34
3	W1830	O1:K1:H7/9	+	−	+	−	−	−	+	+	+	−	−	Chicken with sepsis	71
4	W1831	O1:K1:H7/9	+	−	+	−	−	+	+	+	+	−	−	Turkey with sepsis	71
5	A1075	O2:K1:H4/9	−	−	−	−	−	+	+	+	+	−	−	Chicken with sepsis	2
6	W3757	O2:K1:H7/9	−	−	−	−	−	−	+	+	+	+	+	Piglet feces	71
7	W1825	O2:K1:H7/9	−	−	+	+	+	+	+	−	−	+	+	Turkey with sepsis	71
8	A1112	O2:K1:H6/30	−	−	−	−	+	+	+	+	+	−	−	Bovine mastitis	2
9	W1827	O2:K1:H−/31	+	−	+	−	−	+	+	+	+	−	−	Chicken with sepsis	71
10	A21	O7:K1:H−/3	+	−	+	−	−	+	+	+	+	−	−	Human NBM	3
11	RS176	O7:K1:H−/3	+	−	+	−	−	+	+	+	+	−	−	Human NBM	3
12	A284	O16:K1:H6/12	+	+	+	−	−	−	+	+	+	+	+	Human UTI	3
13	RS226	O18:K1:H7/6	−	+	+	+	+	−	+	+	+	+	+	Human feces	3, 19
14	IHE3034	O18:K1:H7/9	−	−	−	+	+	+	+	−	−	−	−	Human NBM	33, 50
15	IHE3036	O18:K1:H7/9	−	−	−	−	+	−	+	−	−	−	−	Human NBM	33, 50
16	W3773	O45:K1:H7/9	+	−	+	−	−	+	+	+	+	−	−	Human feces	65
17	W3770	O45:K1:H7/9	+	−	+	−	−	+	+	+	+	−	−	Chicken with sepsis	65
18	W3793	O45:K1:H7/9	−	−	−	−	−	+	+	+	+	−	−	Chicken with sepsis	65
19	W3798	O45:K1:H7/9	−	−	−	−	−	+	+	+	+	−	−	Duck with sepsis	65
20	A1521	O83:K1:H?/32	−	−	−	+	+	−	+	+	+	−	−	Human NBM	4
21	764	O18:K5:H5/11	+	+	+	−	+	−	+	+	+	+	+	Human feces	50
22	2980	O18:K5:H5/11	+	+	+	−	+	−	+	+	−	+	+	Human feces	50
23	RS505	O75:K100/11	−	−	−	−	−	+	+	−	+	−	−	Human feces	34
24	RS502	O75:K100/11	−	−	−	−	−	−	+	−	+	−	−	Human feces	34

[a] NBM, newborn meningitis; UTI, urinary tract infection.

strains are given in Table 2. Genomic DNA was isolated and cleaved with *Xba*I, which cuts *E. coli* DNA very rarely. The fragments were separated by OFAGE, and the distinct restriction fragment patterns were compared (Fig. 2A, 3A, and 4A and B).

The two O1:K1/OMP5 isolates resembled each other, as did the two OMP9 strains of this serotype (Fig. 2A, tracks 1 to 4). Similarity of restriction fragment pattern was also seen among the O18:K5 (Fig. 3A, tracks 9 and 10) and the O75:K100 (Fig. 3A, tracks 11 and 12) strain pairs, but all these pairs differed from each other. The three O18:K1 isolates (Fig. 3A, tracks 1 to 3) had some common fragments. In contrast, the O45:K1 isolates W3773 and W3770 (Fig. 3A, tracks 4 and 5) displayed a highly conserved restriction fragment pattern, while the two other strains of this serotype (Fig. 3A, tracks 6 and 7) differed from them as well as from each other. Furthermore, for the O2:K1 strains (Fig. 2A, tracks 5 to 9), markedly different *Xba*I patterns were observed between the different OMP types. The two isolates of serotype O7:K1 showed unrelated *Xba*I patterns (Fig. 2A, tracks 10 and 11). The O16:K1 strain (Fig. 2A, track 12) and the O83:K1 strain (Fig. 3A, track 8) were each unique. Thus, the *Xba*I pattern distinguished between different clonal types and, except for a few cases, between strains of one clonal type. This divergence at the DNA level is also reflected by differences in the virulence patterns (Table 2). Strains with related fragment patterns also displayed similar virulence patterns.

Long-range DNA mapping with virulence-associated gene probes. In order to assign the specific virulence gene clusters to distinct *Xba*I fragments, Southern hybridizations were carried out with the same DNA probes as those used for DNA-DNA dot blots (Fig. 2B to F and 3B to F). The results are summarized in Fig. 4A and B and show that in addition to the observed restriction fragment length polymorphism, the hybridization pattern is also highly heterogeneous between the different strains.

In only a few cases did the DNA probes hybridize with fragments of identical size. The two O45:K1 strains with a highly related *Xba*I pattern yielded comparable hybridization patterns with the *pap*-, *aer*-, and *fim*-specific gene probes (Fig. 3B, D, and F; Fig. 4B, tracks 4 and 5). The hybridization signal for the *aer* probe occurred with clone-specific fragments for three of the O2:K1 strains and the two O7:K1 isolates (Fig. 2B and 4A, tracks 5, 6, 8, 10, and 11). Similarly, the two O1:K1 isolates (Fig. 2D and 4A, tracks 3 and 4) showed *pap*-specific fragments of the same size, as did the O18:K5 strains (Fig. 3D and 4B, tracks 9 and 10). It is interesting that the *pap*- and *hly*-specific DNA probes hybridized with the same fragments. This can be observed for strains of serotypes O2:K1 (Fig. 2C and D and 4A, track 7), O16:K1 (Fig. 2C and D and 4A, track 12), and O18:K1 (Fig. 3C and D and 4B, track 1), arguing for a physical linkage of these gene clusters, which has recently been described for some extraintestinal *E. coli* isolates (8, 26, 27).

DISCUSSION

In this report, we have presented data on virulence patterns among 127 *E. coli* strains with the capsule types K1, K5, and K100. These bacteria had formerly been assigned to clonal types which are highly homogeneous for numerous stable properties, including diverse isoenzymes and outer membrane properties (3, 4). The 35% of the isolates that carried the hemolysin (*hly*) gene cluster were usually hemolytic, as reported by other authors (6, 28). In our collection, only two nonhemolytic strains did not give a positive hybridization signal for *hly* and only one hemolytic strain did not react with the *hly* probe, possibly reflecting production of a cytolysin genetically unrelated to the hemolysin.

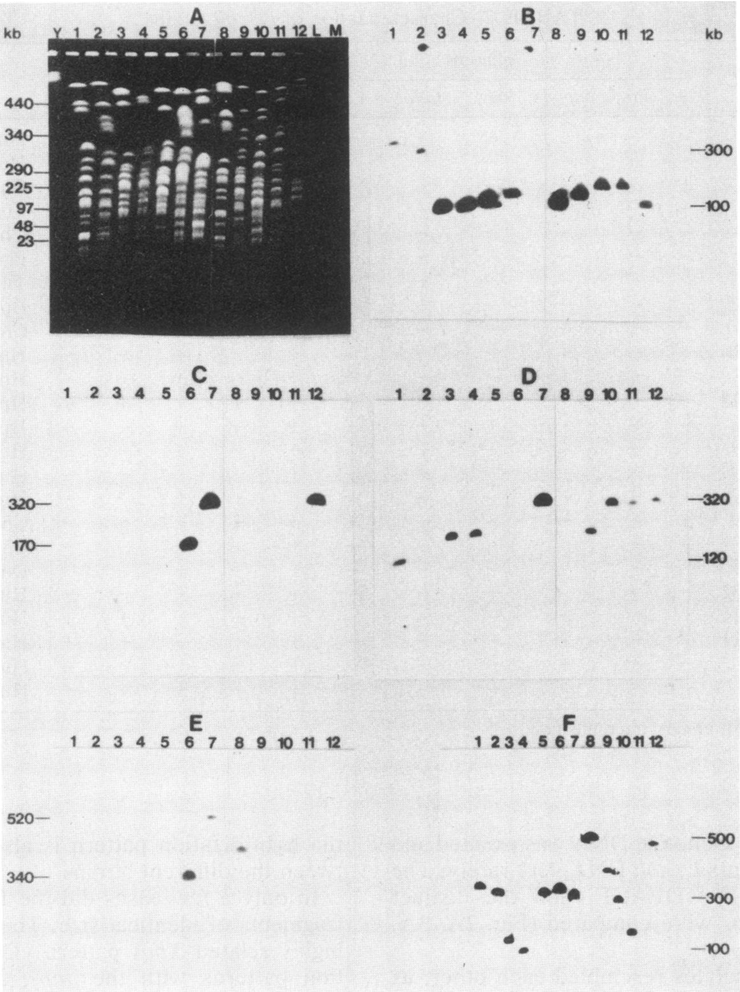

FIG. 2. OFAGE of *Xba*I-cleaved genomic DNAs (A) and Southern hybridization with DNA probes specific for *aer* (B), *hly* (C), *pap*/*prs* (D), *sfa*/*foc* (E), and *fim* (F). The strains in tracks 1 through 12 correspond to those numbered 1 through 12, respectively, in Table 2. Yeast chromosomes (Y), lambda concatemers (L), and *Hin*dIII-cleaved lambda DNA (M) were used as DNA size markers.

In contrast to hemolysin, occurrence of the genetic loci specific for aerobactin and P, Prs, S, and type I fimbriae was much more common than expression of the corresponding virulence factors. For aerobactin, 91 of 102 strains giving a positive hybridization signal expressed an aerobactin-positive phenotype, as also reported by others (11, 28, 49). The strains which are genetically aerobactin positive but phenotypically negative belonged to the serogroups O1:K1, O75:K100, O18:K1, and O45:K1. One strain of serotype O18:K5 was positive in the bioassay but not in the hybridization test, which could be due to the expression of another siderophore.

The discrepancy between genotype and phenotype was more marked for fimbrial adhesins. Of 127 strains tested, 47 carried *sfa* determinants but only 25 showed S-specific binding. The strains able to recognize S-specific receptor structures belonged to serotypes O2:K1, O18:K1, and O83:K1. Gene sequences homologous to *sfa* were also detected on O18:K5/OMP11 strains. As shown previously, the *sfa* determinant belongs to a family of fimbria gene clusters which includes the *foc* determinant, coding for F1C fimbriae, and the *sfr* determinant, encoding S/F1C-related fimbriae (50–52, 55). These determinants show high genetic homology to each other but are different in their receptor specificities.

Of the 77 strains which carried *pap* and *prs* sequences, 52 showed P-specific binding while 26 expressed Prs-specific agglutination. A shift in receptor specificity from Pap to Prs (P-related sequence) (40) on one side accounts for the low incidence of P agglutination found among *pap*-positive isolates. On the other side, the presence of *prf* gene clusters, which code for P-related fimbrial adhesins that are able to bind to tubulus cells but do not recognize P or Prs receptor molecules, was recently shown for O6:K15 and O18:K1 strains (19, 22). Interestingly, O18:K5 strains and O16:K1 isolates display both P- and Prs-specific adherence.

We note that the selection of DNA probes for discrimination between *sfa* and *pap* sequences is very important. Probes which include parts of the regulatory regions of both determinants are not specific enough, as these regions show a high degree of sequence homology (15, 52). Therefore, the probes used in the past (6) were not suitable for studies on the distribution of these virulence gene clusters.

All of our strains carried genes encoding type I fimbriae, but only 61% exhibited type I fimbria-mediated mannose-

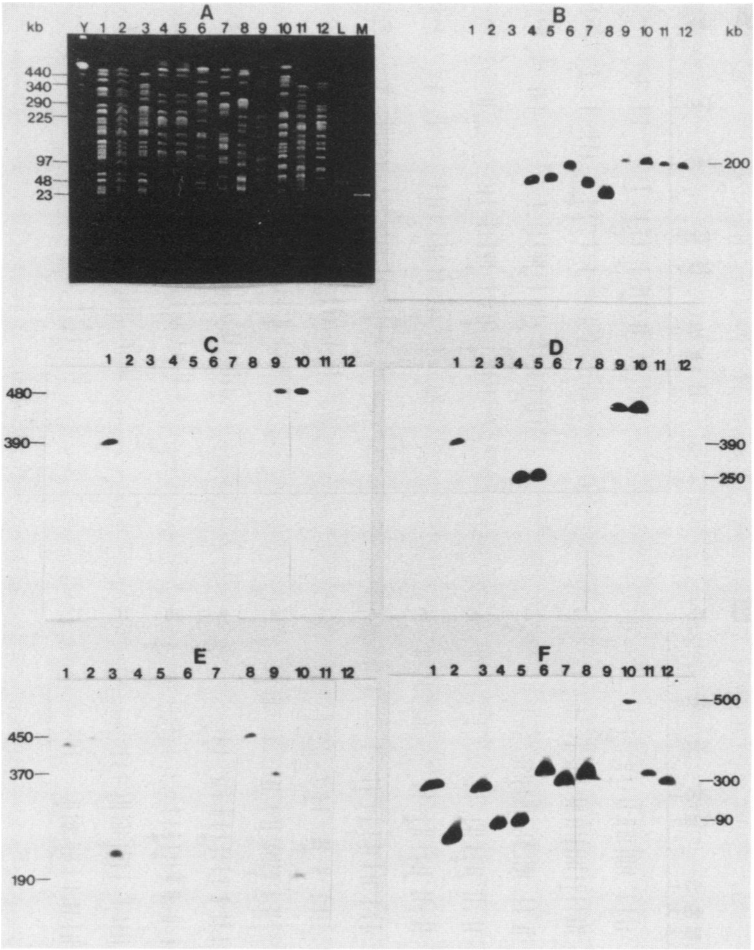

FIG. 3. OFAGE of *Xba*I-cleaved genomic DNAs from 12 additional strains. As in Fig. 2 except that lanes 1 to 12 correspond to strains 13 through 24, respectively, of Table 2.

sensitive agglutination. This might be due to phase variation leading to nonfimbriated bacterial populations, as reported recently (1, 30). Taken together, the results obtained for phenotype versus genotype in fimbrial expression allow us to conclude that in some cases the failure to detect a particular phenotype is due to a shift in receptor specificity (51, 55, 68). On-off switching, first described for type I fimbriae (1, 30), may further contribute to the failure of phenotypic detection. It is also possible that the lack of expression is due to an altered gene structure. Deletions of parts of the gene clusters, or rearrangements and even point mutations, would abolish expression, although in hybridization studies these signals would be detected.

Among some isolates of certain serotypes, the virulence pattern was homogeneous. These strains (O1:K1, O45:K1, O18:K5, and O75:K100) also exhibited related *Xba*I fragment patterns. The virulence patterns therefore do reflect close genetic relatedness of some degree. This relatedness, however, is at a different level than that measured by clonal analyses, based on the analysis of electrophoretic types and OMP patterns, as there was so little correlation of the results from these different methods. The data presented here show that long-range DNA mapping can distinguish individual strains within certain clonal groups and might be useful for fine epidemiological analyses. Recently, Arbeit et al. (5) demonstrated the usefulness of pulsed-field electrophoresis for epidemiological studies of extraintestinal *E. coli* isolates. Our data confirm that this technique can be a powerful tool for evaluation of the genetic relatedness of strains. The use of DNA probes in Southern hybridization studies of long-range separated genomic fragments represents a refinement of this method, leading to unequivocal results on genome structure and the location of virulence determinants.

The distribution of virulence factors among strains belonging to identical clones may be independent of their source of isolation. Thus, O18:K5 strains isolated from urinary tract infections, from cases of newborn meningitis, and from human feces all showed a similar virulence pattern. Also, the virulence genes of O45:K1 strains which have been isolated from human and animal sources did not differ significantly. Both groups yielded similar restriction fragment patterns, indicating their homogeneity. The differences in the virulence pattern among clonal groups may reflect the location of the virulence genes. It has been speculated that the location of virulence gene clusters on plasmids (e.g., aerobactin determinants [37, 67]) or on large unstable chromosomal DNA islands (gene clusters for hemolysin and P fimbriae [19, 22]), which both tend to be lost, may be one reason for the variation of virulence markers among certain groups of strains.

How do the results presented here contribute to or detract from the clonal concept? From experiments in an animal

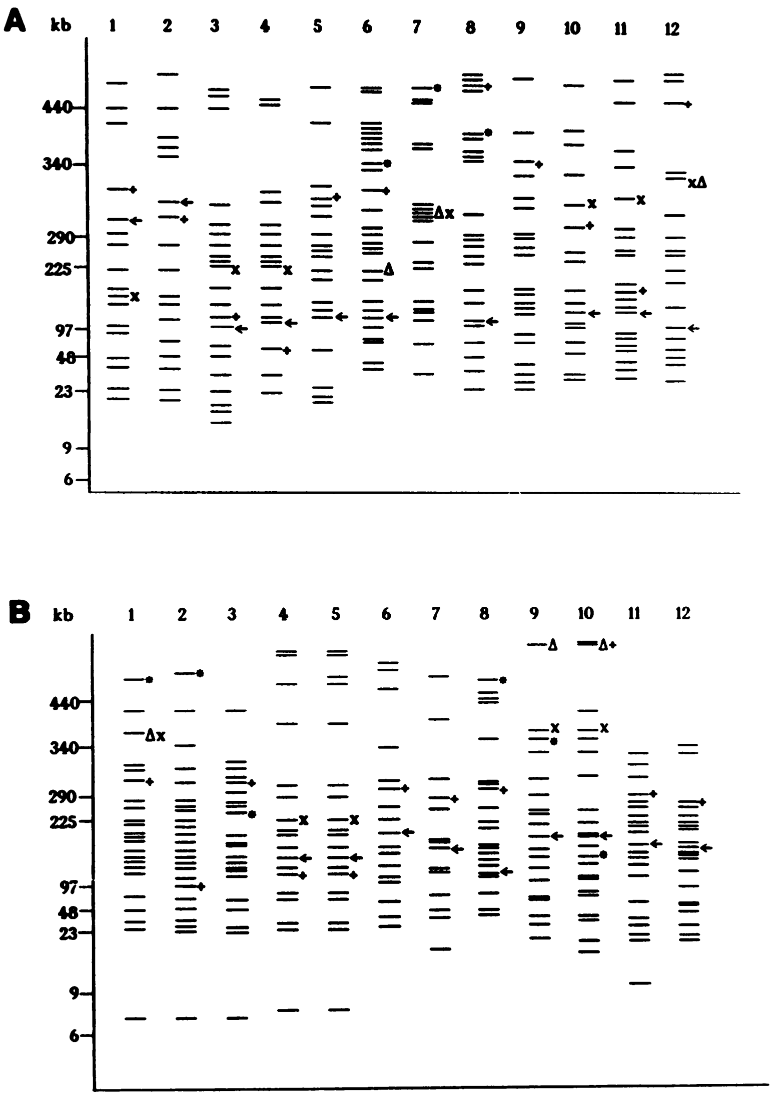

FIG. 4. Interpretation scheme for *Xba*I hybridization patterns. The strains in panel A, lanes 1 to 12, correspond to strains 1 through 12, respectively, in Table 2 (see Fig. 2). The strains in panel B, lanes 1 to 12, correspond to strains 13 through 24, respectively, in Table 2 (see Fig. 3). The fragments hybridizing to individual gene probes are marked as follows: ←, *aer*; Δ, *hly*; ×, *pap*/*prs*; *, *sfa*/*foc*; +, *fim*.

disease model, it has been concluded that virulence is not a clonal property but rather is variable between different members of a clone (4). The results presented here extend those observations by showing that some virulence factors are relatively stable markers for special groups of strains. These are the *pap* determinants, expression of P fimbriae and aerobactin production by O7:K1 and O16:K1 strains, the *sfa* gene clusters within O83:K1 isolates, and the expression of P fimbriae and F1C fimbriae and aerobactin production by O18:K5 isolates (Tables 1 and 2). In most cases, however, strains of a single clonal group showed different virulence patterns (Table 2). In contrast, the restriction fragment length polymorphism and hybridization patterns with virulence-associated gene probes were even more variable and showed only minimal conservation with clonal groups. The latter techniques therefore represent potentially powerful tools for finer epidemiological analysis of the distribution of pathogenic agents among particular populations and in the environment.

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