INFECTION AND IMMUNITY, Oct. 1988, p. 2601–2609
0019-9567/88/102601-09$02.00/0

Vol. 56, No. 10

Isolation and Comparison of *Escherichia coli* Strains from Canine and Human Patients with Urinary Tract Infections

DAVID A. LOW,[1]* BRUCE A. BRAATEN,[1] GERALD V. LING,[2] DEEDRA L. JOHNSON,[2] AND ANNETTE L. RUBY[2]

Department of Pathology, University of Utah Medical Center, University of Utah, Salt Lake City, Utah 84132,[1] and Department of Medicine, School of Veterinary Medicine, University of California, Davis, California 95616[2]

Received 19 February 1988/Accepted 7 July 1988

We analyzed *Escherichia coli* strains isolated from dogs with urinary tract infections (UTIs) in an attempt to determine if any of these strains were similar to *E. coli* isolated from humans with UTIs. Using genotypic and phenotypic traits, we identified four canine and six human *E. coli* UTI isolates that all appeared to be closely related or identical. All isolates shared similar DNA sequences for pyelonephritis-associated pili (*pap*), α-hemolysin (*hly*), and insertion sequence 5 (IS5), on the basis of Southern blot analysis. Similar outer membrane protein, pilin, and plasmid profiles were obtained for each of the isolates, although minor heterogeneity was observed. All of these isolates expressed a neuraminidase-sensitive binding phenotype in contrast to the majority of human isolates, which are known to express an adhesin that recognizes terminal digalactoside residues. Taken together, these results suggest that similar *E. coli* uropathogens may be capable of infecting both dogs and humans. To determine if the intestinal tracts of dogs were a reservoir for uropathogenic *E. coli*, eight paired rectal and urine *pap*$^+$ *E. coli* strains were cultured from dogs with UTIs. By using the same genotypic and phenotypic criteria described above as a basis for strain identity, seven of eight urine-rectal pairs showed intrapair identity. However, each urine-rectal pair displayed a unique overall profile and could be distinguished from the other pairs. We conclude that the uropathogen colonizing the bladders of dogs can also be the predominant strain colonizing the intestinal tracts.

Escherichia coli is the major causative agent of urinary tract infections (UTIs) in both humans (1) and dogs (25). It has been hypothesized that the major reservoir for uropathogenic *E. coli* in humans is the large intestine (46) and that colonization of the vaginal introitus via fecal contamination may precede infection of the bladder (11, 44). It is now clear that at least some uropathogenic *E. coli* strains express gene products that appear to be directly involved in the colonization of the urinary tract. These gene products, which include an adhesin and a pilus, are encoded by the pyelonephritis-associated gene (*pap*) cluster (32, 33). Most uropathogenic *E. coli* strains containing a *pap* operon express an adhesin (P adhesin) directed against the digalactoside portion of globoside (36), although specificities to glycophorin (45) and sialic acids (22, 30) have been described. At least 90% of pyelonephritogenic *E. coli* strains contain *pap*, whereas *E. coli* strains isolated from women without prior UTIs carry *pap* at a lower frequency (36). By using a mouse model, two research groups have obtained evidence that indicates that the P adhesin facilitates the colonization of the renal pelvis leading to pyelonephritis (15, 34).

Although uropathogenic *E. coli* strains that cause human UTIs have been the subjects of intense analysis, *E. coli* strains causing canine UTIs have only recently been studied. Westerlund et al. (49) analyzed 33 *E. coli* UTI isolates from dogs and concluded that the expression of P adhesin and α-hemolysin might contribute to virulence (49). In this paper, we present data showing that many *E. coli* UTI isolates from dogs contain genes homologous to *pap* and to the genes encoding α-hemolysin (*hly*), as evidenced by DNA hybridization. We show that the chromosomal locations of *pap* and, to a lesser extent, *hly* and IS5 (insertion sequence 5) are highly variable, which provides a means by which to compare different *E. coli* strains. Using this genetic analysis in combination with phenotypic markers, such as the plasmid profile, outer membrane protein (OMP) profile, and binding specificity, we have constructed a fingerprint for *E. coli* isolated from dogs and humans with UTIs. Our results indicate that closely related or identical *E. coli* strains can be isolated from both canine and human patients with UTIs. We also present evidence that suggests that dogs can be intestinally colonized with the same *pap*$^+$ *E. coli* strains that infect their urinary tracts.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* isolates from the urine samples of women with cystitis and from the stool samples of women without a history of previous UTI were a gift from Tom Stamey, Stanford University, Stanford, Calif. (44).

E. coli strains were isolated from the urine samples of canine patients at the Veterinary Medical Teaching Hospital, University of California, Davis. Urine specimens were collected from dogs by antepubic cystocentesis (24). Rectal *E. coli* isolates were collected by first cleansing the anal region and then inserting cotton-tipped swabs 3 to 4 in. (1 in. = 2.54 cm) into the rectum. Swabs were streaked onto MacConkey agar plates and incubated in air at 37°C. Bacteria were identified as *E. coli* by standard methods (8).

Paired urine and rectal *E. coli* isolates were obtained from 20 dogs. Dogs admitted to the Veterinary Medical Teaching Hospital were routinely screened by bacterial culture of urine samples and antepubic aspiration for UTI. Rectal isolates were collected from each of these dogs as described above. For each dog, an *E. coli* isolate from the urine sample and an isolate from the rectum were analyzed. Seven paired *E. coli* isolates, including pairs 3, 4, 5, and 7, were from male dogs, and the other pairs were from female dogs. Normal

* Corresponding author.

fecal isolates (total, 54) from dogs without prior UTI history were collected as described above. All specimens were collected from dogs residing in California, except that the pair 2 samples were from an Oregon dog. All *E. coli* strains were stored lyophilized in 20% skim milk or were frozen at −70°C in 30% glycerol–Luria-Bertani broth (LB broth) (29).

Hemolysin, P adhesin, and serotype. Hemolysin activity was determined by plating bacteria onto CM-55 blood agar base medium (Oxoid Ltd., London, England) containing 5% sterile defibrinated sheep blood (26). P-adhesin activity was determined as described previously (26) with P-latex bead agglutination. O serotypes were determined as described previously (13) with antisera obtained from R. A. Wilson, Pennsylvania State University, University Park.

OMP profile. Outer membranes were isolated by the method of Achtman et al. (2), based on the Sarkosyl-insoluble nature of most outer membrane proteins. Briefly, bacteria were harvested from LB broth cultures (100 ml each) at a density of 4×10^8 cells per ml, rinsed once by centrifugation in 10 mM Tris hydrochloride (pH 8.0), and sonicated with a microprobe (model W-220F sonicator; Ultrasonics Inc.). Cell breakage was efficient only if the bacteria were harvested at or below a density of 4×10^8 cells per ml. Whole cells were removed by centrifugation (1,000 × *g* for 20 min at 4°C), and the supernatant solutions were transferred to centrifuge tubes (model 339574; Beckman Instruments, Inc., Fullerton, Calif.). Membranes were collected by centrifugation at 23,800 rpm for 60 min (4°C) with a Ty65 rotor in a Beckman L8-80 ultracentrifuge. Pellets were suspended in 150 μl of water and extracted with Sarkosyl as described previously (2). OMPs were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 10% acrylamide-4% urea gels. Proteins were detected by using Coomassie brilliant blue staining (42). On the basis of the number and size of the OMPs, each OMP profile was given a class designation. Analysis of 26 *E. coli* UTI isolates from dogs and five *E. coli* UTI isolates from humans (see Fig. 2 and 4) showed that each isolate could be assigned to one of six different OMP classes (classes I through VI).

Pilin immunoblotting analysis. Bacteria were serially passaged on LB agar medium before analysis. Bacterial colonies were harvested with wooden toothpicks and suspended directly in 2× Laemmli sample buffer (23), and the bacterial suspensions were immediately boiled for 10 min. After centrifugation for 5 min at 16,000 × *g*, 30 μl of supernatant solution (derived from about 10^8 bacteria) from each tube was loaded onto two 12% acrylamide gels run in parallel, and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After electrophoresis, one gel was stained with Coomassie brilliant blue to visualize protein molecular weight standards (LMW standards; Bio-Rad Laboratories, Richmond, Calif.). Pilin proteins were visualized by immunoblotting as follows. Proteins were electrophoretically transferred to nitrocellulose filters as previously described (27). Filters were first rinsed in TSEN buffer (50 mM Tris hydrochloride [pH 7.5], 0.15 M NaCl, 0.1% [vol/vol] Nonidet P-40, 0.5 mM EDTA) and then rinsed with TSEN buffer containing 2% (wt/vol) bovine serum albumin (fraction V). Nitrocellulose filters were then exposed to anti-pili antiserum (1:500 to 1:1,000 dilution) in TSEN buffer–1% bovine serum albumin overnight at 25°C with rocking. The antiserum used in these experiments was raised in rabbits to pili isolated from strain HB101(pHU845) (35). The pHU845 plasmid contains a *pap* gene cluster from the *E. coli* pyelonephritis isolate J96. Pili were purified as described previously (35). Filters were rinsed in TSEN buffer, incubated with ^{125}I-protein A (Amersham Corp., Arlington Heights, Ill.) for 2 h at 25°C, rinsed again in TSEN buffer, and exposed to XAR-5 film (Eastman Kodak Co., Rochester, N.Y.).

Slide hemagglutination. Bacteria were harvested from tryptic soy agar (TSA) plates and suspended in phosphate-buffered saline (PBS) (50 mM sodium phosphate [pH 7.4], 0.1 M NaCl) containing 2% (wt/vol) α-methylmannoside (αMM) to an optical density at 550 nm of 1.5. Bacterial suspensions (2.5 μl) were mixed with type A human erythrocytes (RBCs) (2.5 μl of a 10% suspension in PBS) and PBS containing αMM (45 μl) and placed on a ringed slide at 4°C. After 0.5 to 1 min of slow rocking, slides were read for hemagglutination. Hemagglutination in the presence of mannose (denoted as mannose-resistant hemagglutination) is used to distinguish type 1 adhesin, which causes a mannose-sensitive hemagglutination (16), from other adhesins that do not recognize a mannose-containing receptor.

Microwell hemagglutination. Bacterial suspensions (optical density at 550 nm of 1.5; 50 μl [see above]) were serially diluted into U-shaped-bottom microtiter wells (Dynatech, Inc., Chantilly, Va) by using αMM (25 μl, 2%) as the diluent. αMM (22.5 μl, 2% in PBS) and RBCs (2.5 μl of 5% RBCs in 2% αMM and 1% bovine serum albumin [fraction V]) were added to each well. After the plates were covered and the suspensions were mixed by gentle agitation, the plates were incubated at 4°C overnight before observation for hemagglutination.

Inhibitors. Sialic acid residues were removed from human RBCs by addition of neuraminidase (from *Vibrio cholerae*; Calbiochem-Behring, La Jolla, Calif.) (0.2 U/ml) for 2 h at 37°C. Neuraminidase-treated RBCs were stored at 4°C and used within 1 day of preparation. Fetuin (type III), asialofetuin, colominic acid, *N*-acetylneuramin-lactose, and *N*-acetylneuraminic acid were from Sigma Chemical Co., St. Louis, Mo.

Plasmid analysis. Plasmids were isolated by an alkaline lysis method developed by Portnoy and Falkow (38). A single bacterial colony was inoculated into 2 ml of LB broth and incubated on a tube roller apparatus (New Brunswick Scientific Co., Inc., Edison, N.J.) overnight at 37°C. Bacterial cells were rinsed once by centrifugation (3,000 × *g*, 10 min) in 50 mM Tris hydrochloride (pH 8.0)–10 mM EDTA and resuspended in 40 μl of this same buffer. Lysis buffer (50 mM Tris hydrochloride [pH 12.42], 10 mM EDTA, 4% sodium dodecyl sulfate) was added to each tube (0.6 ml per tube). After mixing by inversion, tubes were incubated at 37°C for 20 min. Neutralization was effected by the addition of 40 μl of 2 M Tris hydrochloride (pH 7.0). After a 5-min incubation at 22°C, 5 M NaCl was added (160 μl) and tubes were incubated for an additional hour at 4°C. Tubes were then centrifuged for 10 min in an Eppendorf 5414 centrifuge (16,000 × *g*), and plasmid DNA was precipitated from the supernatant fraction by the addition of isopropanol (0.55 ml per tube). Plasmid DNA was analyzed by electrophoresis with 0.7% agarose gels in Tris borate buffer (24).

Preparation of DNA probes. Four DNA fragments were used in this study (Fig. 1). DNA restriction fragments were separated on agarose and polyacrylamide gels as described previously (28). Specific DNA fragments were isolated and purified by electroelution and labeled with ^{32}P by nick translation with [^{32}P]dCTP as previously described (39).

Two α-hemolysin (*hly*) DNA fragments, both derived from plasmid pSF4000 (48), were used in Southern DNA hybridization analyses. The 6.1-kilobase-pair (kb) *Aval* DNA fragment contained most of the region required for Hly expres-

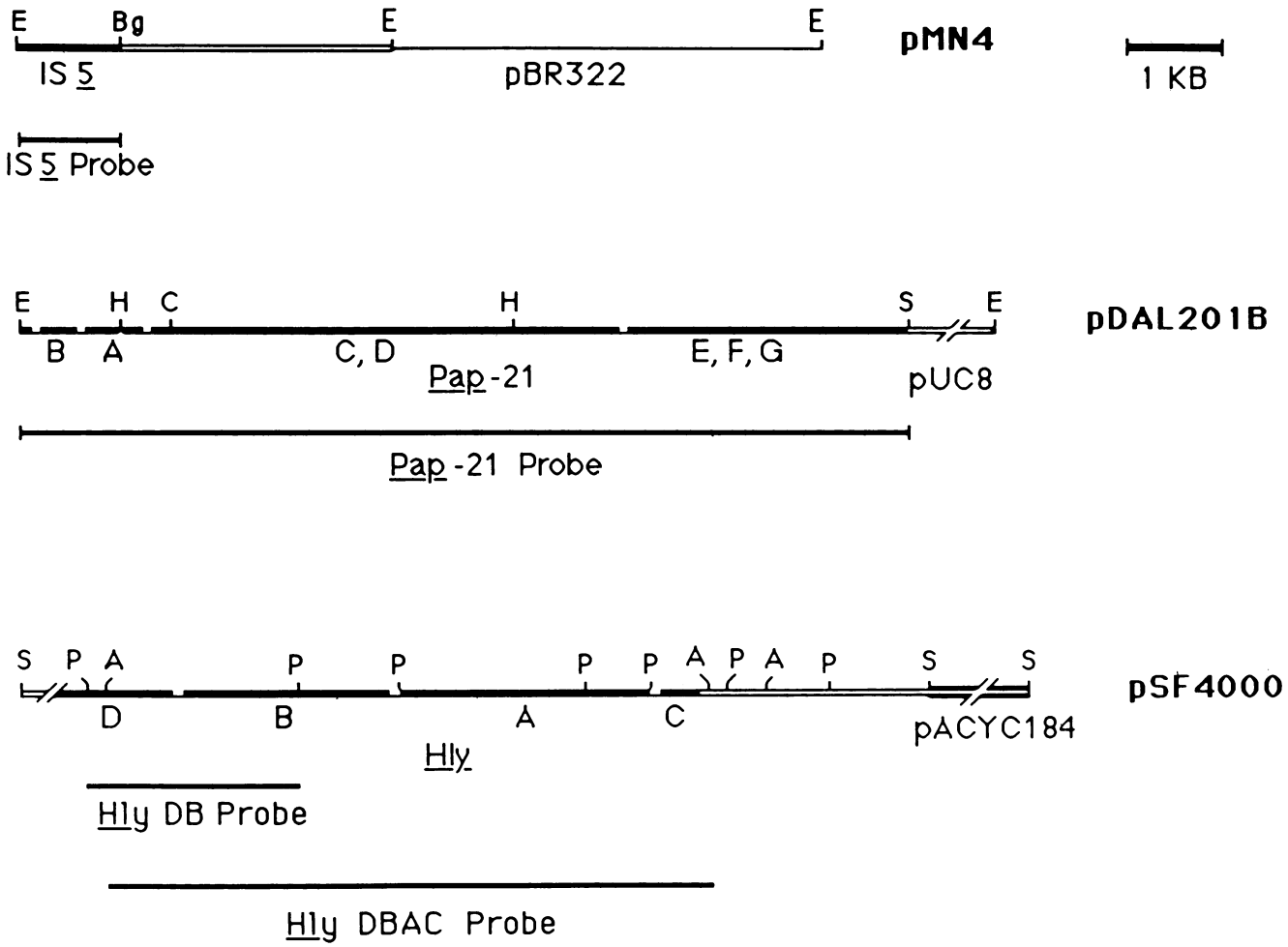

FIG. 1. DNA fragments used as probes for identification of *pap*, *hly*, and IS*5*. DNA fragments from each of the plasmids shown were isolated and labeled with [^{32}P]deoxynucleotide triphosphates as described in Materials and Methods. These labeled DNA fragments were used as DNA hybridization probes for both Southern (43) and colony (14) DNA blotting as described in Materials and Methods. The genes are shown below the fragments, and the restriction endonuclease recognition sites are shown above the fragments. Abbreviations: E, *Eco*RI; Bg, *Bgl*II; H, *Hin*dIII; C, *Cla*I; S, *Sal*I; P, *Pst*I; A, *Ava*I.

sion (*hlyDBAC* [10]). The 2.2-kb *Pst*I DNA fragment (*hlyDB*) contained portions of *hlyB* and *hlyD* (10). Both *hly* DNA probes were internal to the genes required for hemolysin expression.

The 0.94-kb *Eco*RI-*Bgl*II DNA restriction fragment from plasmid pMN4 (12) was used as a DNA probe for detection of insertion sequence 5 (IS*5*). This DNA restriction fragment spans most of the 1,195-base-pair IS*5* element.

The 9-kb *Eco*RI DNA fragment from pDAL201B (27) was used to detect DNA sequences which share homology with *pap*. This DNA fragment contains almost all of the *pap* operon from the pyelonephritis strain C1212 (27). This DNA fragment confers a pilus-positive, P-adhesin-positive phenotype to *E. coli* HB101. The latter strain does not express pili or P adhesin (18).

DNA hybridization. Chromosomal DNA was isolated as previously described (18). Following cesium chloride gradient centrifugation (28), DNA samples were dialyzed against TE buffer (10 mM Tris hydrochloride [pH 8.0]–1 mM EDTA) before digestion with restriction endonuclease *Hin*dIII. DNA restriction fragments were separated by electrophoresis on 0.7% agarose gels in 0.04 M Tris acetate–1 mM EDTA buffer. DNA hybridizations were performed by the method of Southern (43) with aminophenylthioether cellulose (41) and nitrocellulose filters (Schleicher & Schuell, Inc., Keene, N.H.). Initial Southern blot analysis with the *pap* DNA probe was performed by using 17 *E. coli* isolates from human patients with UTIs (6 strains were P adhesin positive; 11 strains were mannose-resistant hemagglutination positive and P adhesin negative) and 26 *E. coli* isolates from canine patients with UTIs (2 strains were P adhesin positive; 24 strains were mannose-resistant hemagglutination positive and P adhesin negative). Colony DNA hybridization was performed as described previously (14, 26).

RESULTS

Comparative analysis of *E. coli* strains causing canine and human UTIs. (i) Southern DNA hybridization. Previous studies have shown that most pyelonephritogenic *E. coli* strains isolated from humans contain the DNA sequences for pyelonephritis-associated pili (*pap*) and hemolysin (*hly*) (36). To determine if *E. coli* isolates from dogs also contained *pap* and *hly* DNA sequences, strains were tested by colony DNA hybridization with both *pap-21* and *hlyDBAC* DNA probes (Fig. 1). The frequencies of carriage of either *hly* or *pap* in *E.*

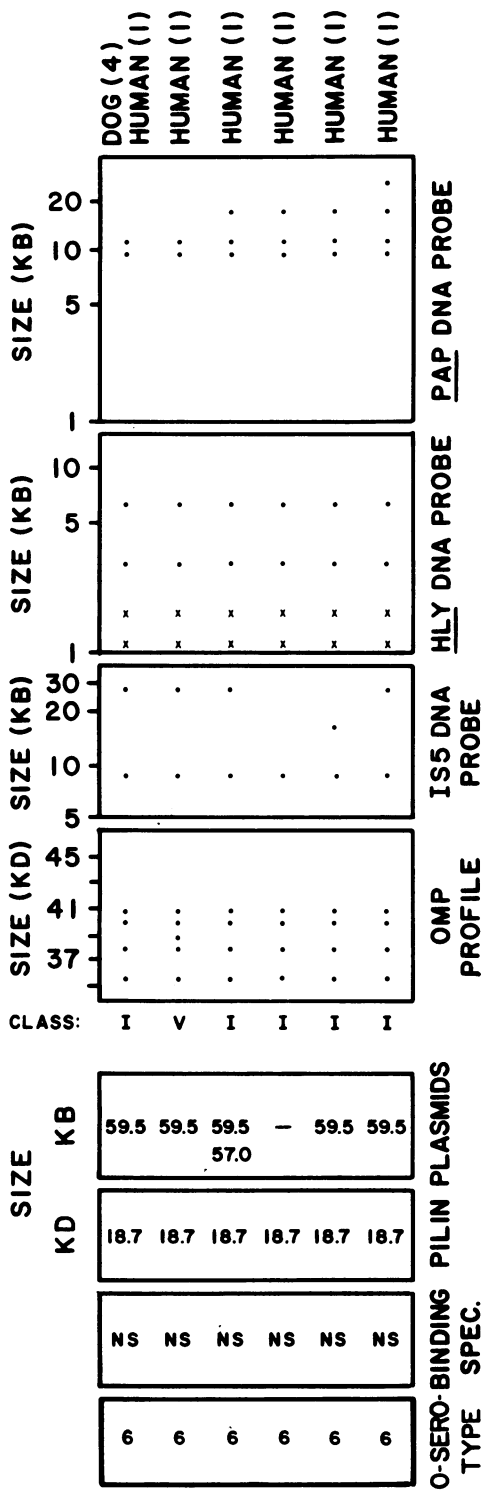

FIG. 2. Summary of genotypic and phenotypic analyses of uropathogenic *E. coli* isolated from dogs and humans. The source of each *E. coli* isolate (human or dog) is shown at the top, along with the number of isolates (shown in parentheses), each obtained from a different patient, expressing the same genotypic and phenotypic traits. The top three panels show the results of Southern blot analyses of the *E. coli* isolates. The DNA probe used is shown at the right, and the sizes of the DNA fragments (in kilobase pairs [KB], logarithmic scale) hybridizing to each probe are shown at the left. The X indicates that the DNA fragment hybridized with both the *hlyDBAC* and *hlyDB* DNA probes. The fourth panel shows the sizes (in kilodaltons [KD]) of the major OMPs obtained from each of the *E. coli* isolates. Each of the OMP profiles was given a class designation as described in Materials and Methods. The fifth and sixth panels show the sizes of plasmids (in kilobase pairs) and pilin monomers (in kilodaltons), obtained from each *E. coli* isolate as described in Materials and Methods. A dash indicates that plasmids were not detected. The seventh panel shows that the binding specificity (BINDING SPEC.) of each *E. coli* isolate was neuraminidase sensitive (NS) (pretreatment of human RBCs with neuraminidase abolished agglutination). The bottom panel shows the O serotype of each *E. coli* isolate.

TABLE 1. Frequency of carriage of *pap* and *hly* DNA sequences in *E. coli* from different sources[a]

Genotype	No. of *E. coli* isolates (%) carrying sequence(s) from dogs	
	With UTIs (n = 108)	Without UTIs (n = 54)
pap$^+$	46 (43)	6 (11)
hly$^+$	62 (57)	6 (11)
pap$^+$ *hly*$^+$	45 (42)	4 (7)

[a] The *pap-21* and *hlyDBAC* DNA probes (Fig. 1) were used in colony DNA hybridization experiments as described in Materials and Methods to determine the frequency of carriage of *pap* and *hly* in *E. coli* isolates from dogs.

coli isolated from dogs with UTIs were four- to fivefold higher than those in dogs without UTIs (Table 1). Most of the *E. coli* strains containing *pap* (45 of 46 strains) also contained *hly* DNA sequences. These data are comparable with results obtained with *E. coli* isolates from humans with UTIs (20, 26, 36).

One question that these data raise is whether the higher incidence of *pap* and *hly* from canine patients with UTIs was due to repeated reisolation of identical *E. coli* strains. Analysis of eight urine-rectal paired *E. coli* isolates (see below) and four additional UTI isolates (all serotype O6 [data not shown]) with *pap*, *hly*, and IS*5* DNA probes and plasmid profiles indicated that all of the isolates, with the exception of urine-rectal pair 2R, were strains distinct from one another. Analysis of 11 of the normal fecal *E. coli* strains by plasmid profiles showed that only two of the isolates shared similar profiles. One of these isolates was *pap*$^+$, and the other was *pap*$^+$ *hly*$^+$ (data not shown). Although these strains represented only a portion of the total strains analyzed, the results suggest that the increased incidence of *pap* and *hly* in UTI isolates from dogs was not solely due to reisolation of a very limited number of *E. coli* clonal variants.

DNA sequences from *pap*, *hly*, and insertion sequence 5 (IS*5*) were used to compare the genetic relatedness of *E. coli* strains isolated from canine and human patients with UTIs. Both *pap* (18) and *hly* (31, 47) appear to be located on the bacterial chromosome in human UTI strains. All of the strains used in the present study contained chromosomally located *pap* and *hly* (data not shown). Normally, most chromosomal DNA sequences would not be useful for strain comparison, because their chromosomal locations are relatively constant. However, the chromosomal locations of the three DNA sequences that we analyzed were highly variable (see below) and thus provided us with a means of comparing different isolates. Southern DNA hybridization results with four canine and six human *E. coli* UTI isolates are shown in

Fig. 2. These strains were chosen for further analysis after initial Southern blot screening of *pap*$^+$ *E. coli* isolates from 26 canine and 17 human patients with UTIs (see above). All of the strains contained two common DNA fragments that hybridized with *pap*. Additional *pap* DNA sequences (one or two) were found in four of the strains analyzed.

All of the strains contained the same four DNA restriction fragments that hybridized to the *hlyDBAC* probe. These four DNA fragments appeared to be shared by almost all of the UTI strains isolated from dogs that we analyzed in this study (see Fig. 4, *hly* DNA probe panel). To help determine what these four DNA sequences represented, we used a smaller *hly* DNA fragment from one end of *hly* (*hlyDB* [Fig. 1]) as a DNA probe. As depicted by the X in Fig. 2, this DNA probe hybridized only to the two smaller DNA fragments. These results suggested that the two smaller DNA fragments were adjoining and located at one end of *hly*. One of these DNA fragments should be completely internal to *hly*, whereas the other fragment would be expected to span the junction of *hly* and the bordering DNA sequence up to the first *Hin*dIII recognition site outside *hly*. Thus, the four *Hin*dIII DNA fragments hybridizing to the *hlyDBAC* probe probably represented one *hly* DNA sequence that contained three internal *Hin*dIII recognition sites. None of the strains contained any additional *hly* sequences.

Two DNA fragments hybridizing to the IS*5* DNA probe were detected in all but two of the *E. coli* isolates. The latter two isolates, both from humans, had the smaller (7.5-kb) DNA fragment. One of these isolates contained an additional DNA fragment (17.5 kb) hybridizing to the IS*5* DNA probe. It seems likely that each band represented a different IS*5* sequence, since *Hin*dIII, which was used in these experiments to digest chromosomal DNA, does not cleave within IS*5* (9).

(ii) OMP profiles. Outer membranes were isolated from each of the strains as described above, and OMPs were analyzed by sodium dodecyl sulfate-polyacrylamide gel electophoresis (Fig. 3C). This type of analysis has been shown to be useful in distinguishing different bacterial clonal types which are defined as bacterial strains that are linearly descended from a common progenitor cell (2, 3). On the basis of the molecular masses of OMPs, OMP profiles were assigned to each bacterial isolate (Fig. 2). Nine of the isolates displayed the same OMP profile, whereas one of the strains expressed an additional OMP of 39 kilodaltons (kDa).

(iii) Plasmid profiles, pilin profiles, and O serotype. Further analysis of the paired *E. coli* strains was done by isolating plasmids from each strain and comparing their molecular weights. Although many plasmids lack the stability of chromosomal markers, they can provide important information about strain relatedness (19). All but one isolate contained a plasmid of 59.5 kb. One isolate contained an additional plasmid of 57 kb.

Although a number of distinct pili immunotypes have been described (35, 37), antisera raised against pili from the pyelonephritogenic strain J96 cross-reacts with many different Pap pili (35). We used this antisera in immunoblotting experiments to determine the size(s) of pilin monomers expressed by each *E. coli* strain. All isolates expressed an 18.7-kDa pilin monomer.

All of the *E. coli* isolates were identified as O serotype 6 (Fig. 2).

(iv) Binding specificities. In addition to encoding pilin monomers, the *pap* gene cluster also encodes separate gene products required for adhesion to epithelial cells (32, 33). Most *pap*-containing uropathogenic *E. coli* strains isolated from humans bind to the terminal digalactoside residues of the P blood group antigen (21, 36). However, although all of the 10 human and canine *E. coli* isolates (Fig. 2) agglutinated human RBCs in the presence of mannose, none of them agglutinated P latex (see above). These results indicated that these *E. coli* expressed an adhesin that did not recognize the P (Gal-Gal) receptor.

To determine if neuraminic acid was a component of the receptor, human RBCs were treated with neuraminidase. This treatment abolished hemagglutination by all of these *E. coli* strains (Fig. 2). However, addition of either fetuin (final concentration, 10 mg/ml), α_1-acid glycoprotein (2 mg/ml), colominic acid (4 mg/ml), *N*-acetylneuramin-lactose (2 mg/ml), or *N*-acetylneuraminic acid (2 mg/ml) did not block hemagglutination (results not shown). Together, these results indicated that the adhesin of these strains recognized sialic acid complexed with additional unknown moieties. We have designated this binding as neuraminidase sensitive (NS [Fig. 2]). These results also suggested that the adhesin specificity of these strains was different from the α-sialyl-β-2,3-galactosyl-specific adhesin (S adhesin) previously described (22, 30), since both fetuin and orosomucoid effectively inhibit hemagglutination by S adhesin (30) but not the NS adhesin described here. However, our results need to be cautiously interpreted, since we have not yet studied the binding properties of the purified NS adhesin or the genes that encode this adhesin.

Together, these results showed that the four *E. coli* UTI isolates from dogs and six *E. coli* UTI isolates from humans shared a number of genotypic and phenotypic properties. One of the *E. coli* isolates from humans appeared to be identical to the four canine isolates by all of the criteria used for analysis.

Comparative analysis of *E. coli* strains isolated from the urine and rectal samples of dogs with UTIs. (i) Southern DNA hybridization. It is commonly assumed that intestinal colonization by *E. coli* precedes UTI. This assumption is based on the fact that *E. coli* makes up a portion of normal gut flora, and the predominant *E. coli* causing UTIs in humans are of the same serotype as normal fecal *E. coli* (40, 46). To determine if canine patients with UTIs were intestinally colonized with the same *E. coli* causing their UTIs, we analyzed paired urine-rectal *E. coli* isolates obtained from dogs with UTIs. As shown below, the variability in chromosomal location of *pap* was an essential part of the fingerprint made for each *E. coli* strain. This limited us to analyzing urine-rectal paired isolates that were both *pap*$^+$. The *pap-21* DNA probe hybridized to both isolates of 9 of the 20 paired urine-rectal *E. coli* strains analyzed; both isolates of eight pairs were negative for hybridization. The results for *pap-21* probe hybridization were mixed for 3 of the 20 pairs (1 isolate positive and 1 isolate negative); these urine and rectal isolates were presumed to be different strains of *E. coli*, since *pap* has a chromosomal location (see reference 18 and below) and should not be easily lost from an isolate as might occur if *pap* was plasmid borne. Eight of the nine *pap*$^+$ paired rectal and urine strains were chosen for the detailed genotypic and phenotypic analysis described below.

Southern DNA hybridization results with four paired urine-rectal *E. coli* isolates are shown in Fig. 3. Hybridization with the *pap-21* DNA probe (panel A) showed that the chromosomal location of *pap* was different for each of the four paired strain sets yet appeared identical for each urine-rectal pair. Analysis with the *hlyDBAC* DNA probe (panel B) showed that pair 6 lacked any DNA sequences homologous to *hly*. The other three pairs probed with *hlyDBAC* DNA

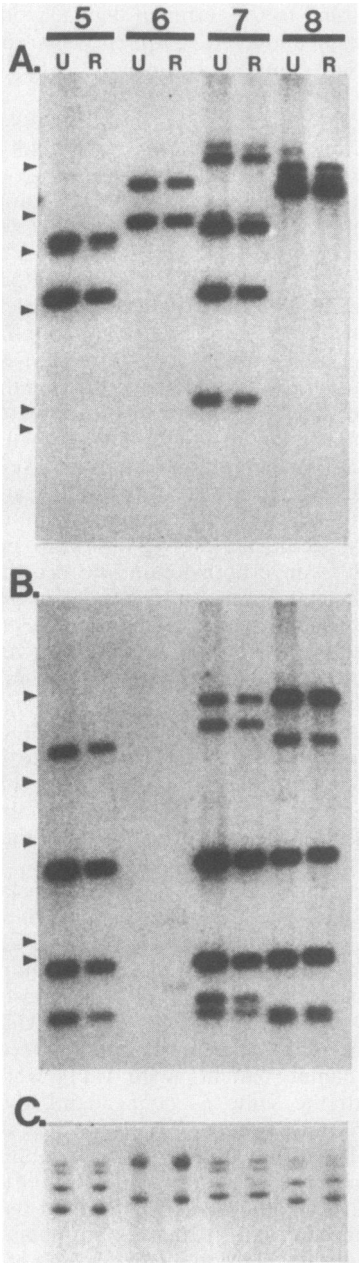

FIG. 3. Southern blot and OMP analyses of paired urine (U) and rectal (R) *E. coli* isolates from dogs. (A and B) Chromosomal DNA from *E. coli* paired isolates 5, 6, 7, and 8 was isolated and digested with restriction endonuclease *Hin*dIII. DNA fragments were separated by electrophoresis on 0.7% agarose gels and transferred to nitrocellulose paper as described in Materials and Methods. DNA hybridization and autoradiography with ^{32}P-labeled *pap* (A) and *hly* (B) DNA fragments (see Fig. 1) was carried out as described above. The arrows on the left show DNA size standards of (bottom to top) 2.0, 2.3, 4.3, 6.7, 9.5, and 23.7 kilobases (kb) in size. (C) Outer membranes from *E. coli* paired isolates 5, 6, 7, and 8 were prepared, and OMPs were analyzed on 10% polyacrylamide-4 M urea gels as described in Materials and Methods. Proteins were visualized by Coomassie brilliant blue staining.

showed identical intrapair but different interpair hybridization patterns.

DNA hybridization results for all eight paired isolates, including results obtained using the IS*5* DNA probe, are

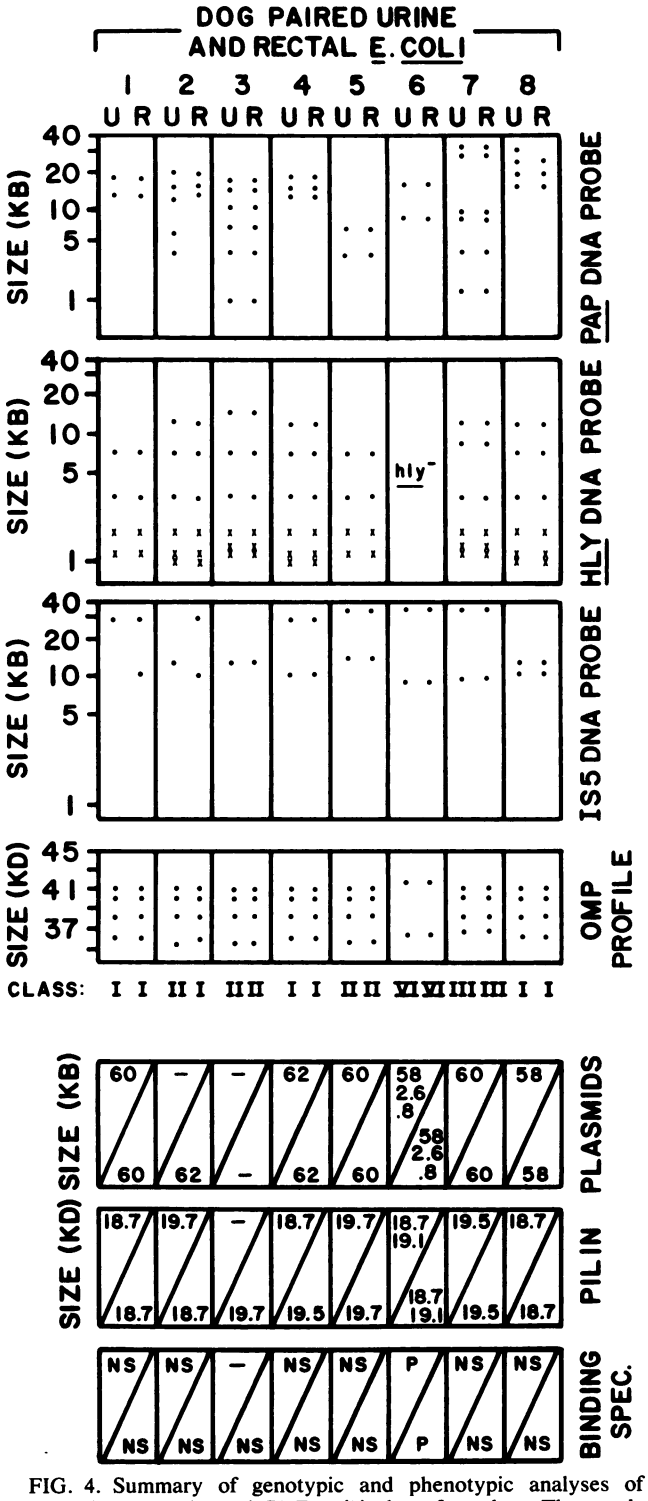

FIG. 4. Summary of genotypic and phenotypic analyses of paired urine (U) and rectal (R) *E. coli* isolates from dogs. The panels are similar to those in Fig. 2. In the second panel (*hly* DNA probe), the DNA fragment hybridized only with the *hlyDBAC* probe (●); the DNA fragment hybridized with both the *hlyDBAC* and *hlyDB* DNA probes (X). A dash indicates that the particular phenotype analyzed (the presence of plasmids, pilin monomers, or adhesins) was not detected. In the bottom panel (BINDING SPEC.), P signifies binding to the P blood group antigen, specifically to terminal Gal-Gal residues (see the text).

summarized in Fig. 4. In seven of the eight paired isolates, the urine and rectal paired strains appeared identical with all three DNA probes with two notable exceptions. The urine isolate of pair 1 lacked one of the two bands present in the rectal isolate of pair 1 after hybridization with the IS*5* DNA probe (Fig. 4). The pair 8 urine and rectal isolates appeared identical, except that one additional DNA fragment with homology to *pap* was observed for the urine isolate (Fig. 3 and 4).

The urine and rectal isolates from one of the eight pairs (pair 2) were distinct from each other, on the basis of *pap-21* and IS*5* DNA probe hybridization (Fig. 4). In addition, OMP, plasmid, and pilin profiles were also different between the urine and rectal isolates of pair 2 (see below). Interestingly, the rectal isolate of pair 2 and urine and rectal isolates of pair 4 appeared to be similar on the basis of *pap*, *hly*, and IS*5* DNA hybridization analyses. Also, these isolates shared common OMP and plasmid profiles (see Fig. 4 and below).

Seven of the eight pairs contained *hly* DNA sequences. All but one of these pairs shared four common DNA fragments that hybridized with the *hlyDBAC* DNA probe (pair 7 lacked the 7.5-kb DNA fragment). Some *E. coli* isolates contained two additional DNA fragments that hybridized to the *hlyDBAC* probe (pair 2, 3, 4, 7, and 8). Pairs 2, 4, and 8 appeared to contain the same two additional DNA fragments. In each pair, one of these DNA fragments hybridized to the *hlyDB* probe (Fig. 4). Thus, it seems likely that the additional two DNA fragments represented another *hly* DNA sequence in the chromosome. In the *E. coli* strains that contained two *hly* DNA sequences, both DNA sequences appeared to contain the same three internal *Hin*dIII recognition sites, since additional DNA fragments hybridizing to the *hly* DNA probe were not found.

(ii) OMP profiles. Four different OMP patterns were seen for the eight paired isolates. The same seven paired urine-rectal isolates that appeared similar by DNA hybridization (above) displayed similar OMP profiles (Fig. 3C and 4).

(iii) Plasmid and pilin profiles. Six of the eight pairs contained a large plasmid (58 to 62 kb), whereas no plasmids were isolated from pair 3. Again, the same seven paired strains that displayed similar DNA hybridization and OMP patterns showed similar plasmid profiles between the urine and rectal isolates of each pair.

Four different pilin sizes were detected, ranging from 18.7 to 19.7 kDa in size (Fig. 4). Pair 2 urine and rectal isolates expressed pilin of different sizes, in agreement with DNA hybridization, OMP, and plasmid profile results, indicating that these two isolates were distinct. The urine isolate of pair 3 did not express pilin monomer, whereas the rectal isolate expressed a 19.7-kDa monomer. This result is not consistent with all other results shown in Fig. 4, which indicated that the pair 3 urine and rectal isolates were very similar. Another discrepancy can be seen with pair 4, in which pair 4 urine and rectal isolates expressed pilin subunits of different size. These two apparent discrepancies are discussed below. The urine isolates in the remaining five pairs each expressed pilin monomers of similar size to their paired rectal isolates.

(iv) Binding specificities. One of the *E. coli* urine-rectal pairs (pair 6) expressed a P adhesin, evidenced by agglutination of P latex beads (Fig. 4). All of the other isolates (with the exception of the urine isolate of pair 3, which also failed to express pilin) expressed what we have designated above as an NS adhesin; agglutination of human RBCs was blocked by pretreatment of the blood cells with neuraminidase.

Together, the results presented in Fig. 3 and 4 indicated that in seven out of eight of the canine patients with UTIs analyzed, the *E. coli* strain infecting the urinary tract was a predominant part of the intestinal *E. coli* flora.

DISCUSSION

The results presented in Fig. 2 indicated that very closely related or identical *E. coli* with distinctive genotypic and phenotypic properties could be isolated from both canine and human patients with UTIs. Previous results indicated that in families that own dogs and cats, some of the *E. coli* electrophoretic types were shared between the human and animal subjects (5). Further characterization of these shared *E. coli* isolates was not pursued. Our results raise the possibility that human-to-dog or dog-to-human transmission of UTI occurs or has occurred in the recent past. An alternative hypothesis is that *E. coli* strains causing canine and human UTIs are subject to similar biologic parameters that select for a clonal population with the specific genotype (and phenotype) that we observed. This hypothesis does not seem as likely, since our results (Fig. 3 and 4) showed that *E. coli* strains causing canine UTIs were heterogeneous with respect to the chromosomal positions of *pap*, *hly*, and IS*5*, in addition to their OMP and pilin phenotypes. Prospective studies with human patients with UTIs who own dogs must be conducted to determine if there is a class of human UTIs that can be zoonotic.

All six human isolates that appeared to be closely related to the canine isolates (Fig. 2) expressed the NS adhesin. It is tempting to speculate that the *E. coli* expressing this NS adhesin are able to bind to both human and canine uroepithelial and intestinal epithelial cells. All but one of the eight *E. coli* urine-rectal pairs expressed the NS adhesin phenotype (Fig. 4). In addition, 16 of 16 additional *pap*$^+$ *E. coli* isolates from dogs, including the four isolates shown in Fig. 2, expressed the NS binding phenotype (unpublished results). Dogs do not appear to express cell surface antigens containing digalactoside (G. Schoolnik and D. Lark, personal communication) and thus might be refractory to colonization by the majority of *E. coli* isolates from humans which express Gal-Gal adhesin (36). Our results differ from those obtained recently for canine UTI in which almost one-half (16 of 33) of the *E. coli* UTI isolates expressed the P adhesin, whereas only 8 of 33 expressed non-P adhesins (49). We do not know the reason for this discrepancy, although it is possible that there might be differential susceptibility of some canine breeds to infection with *E. coli* expressing P adhesin.

It seems likely that the NS adhesin is encoded by a DNA sequence with homology to *pap* since we found that almost all canine *E. coli* UTI isolates that were *pap*$^+$ were also NS adhesin positive and expressed pili that cross-reacted with anti-Pap pili antiserum (Fig. 4). The one exception was *E. coli* isolate 3U, which was *pap*$^+$ and NS adhesin negative and pili negative. However, the *pap* genes will have to be cloned and transformed into pili- and adhesin-negative *E. coli* to determine if they encode adhesin with NS specificity.

The data presented here (Fig. 3 and 4) support the conclusion that, in many cases, colonization of the urinary tracts of dogs by *E. coli* was accompanied by intestinal colonization by the same uropathogen. However, two assumptions must be made in order for this conclusion to be valid. The first assumption is that rectal swabs provided a good estimation of the intestinal flora. Supportive evidence for this has been reported previously (4). The second assumption is that there was not any contamination of the rectal sample with urine. This is a potentially serious problem with human

female patients. However, the anatomy and posture of the dog (especially for male dogs) makes cross-contamination highly unlikely, especially after cleansing of the anal region prior to taking a rectal sample.

The colonization that we observed must have been extensive, since only a single *E. coli* isolate was chosen at random for analysis from the rectum of each dog, and in seven out of eight instances, the rectal *E. coli* appeared identical or nearly identical to the *E. coli* isolated from the bladder. Both dogs and humans normally carry a diverse population of intestinal *E. coli* strains which have been classified as either transient or resident strains on the basis of the number of times these strains can be isolated over a period of time (4, 6). Most of this diversity appears to be generated by incoming strains, rather than recombination between strains residing within a single host (4). We do not know if the bladder or intestine (or some other site) is colonized first by uropathogenic *E. coli*. However, it appeared that by the time of infection of the urinary tract of the dog the intestinal *E. coli* flora changed dramatically as a result of takeover by the same uropathogenic *E. coli* colonizing the bladder.

In two dogs, we found one genotypic change (pairs 1 and 8 [Fig. 4]), and in one dog, we found one phenotypic change (pair 4) between the paired urine and rectal isolates. The urine isolate of pair 1 lacked a DNA fragment hybridizing to IS*5*, and the urine isolate of pair 8 contained an additional DNA fragment hybridizing to the *pap* DNA probe. The urine and rectal isolates of pair 4 expressed different size pilin monomers. It is possible that the genotypic and phenotypic changes observed occurred in transit from the intestine to the bladder or vice versa. Alternatively, these changes might have occurred after isolation of the bacteria from the dogs. Similar genetic changes have been observed to occur in stab cultures stored for long periods of time (17). In any case, these results suggest that *pap* is (or was recently) a moveable genetic element similar to insertion sequences and transposons. This would explain the diverse array of *pap* locations found in the *E. coli* strains analyzed here.

Finally, the results that we present here suggest the possibility that humans, as well as dogs, may be intestinally colonized with the same uropathogenic *E. coli* that is causing infection of the urinary tract in that patient. If this is correct, then at least some UTIs in humans should be viewed not as an isolated infection of the urinary tract but as an infection of the intestinal tract as well. In addition, previous results have suggested that colonization of the vaginal introitus may precede bladder colonization (11, 44). The definition of the host factors that determine susceptibility to both intestinal and urinary tract colonization is one area of research that needs further exploration. Previous work has indicated that the epithelial cell receptors for bacterial adhesins might play some role in susceptibility to UTIs (7, 11). It will be important to determine what role, if any, intestinal colonization plays in the initiation of human UTIs and what factors determine the susceptibility of a host to colonization by uropathogenic *E. coli*.

ACKNOWLEDGMENTS

We thank David Lark for discussions which initiated this study.

This work was supported by American Cancer Society grant MV278 and Public Health Service grant AI23348 from the National Institutes of Health to D. Low.

LITERATURE CITED

1. **Abraham, E., B. E. Brenner, and R. R. Simon.** 1983. Cystitis and pyelonephritis. Ann. Emerg. Med. **12**:228–234.
2. **Achtman, M., A. Mercer, B. Kusecek, A. Pohl, M. Heuzenroeder, W. Aaronson, A. Sutton, and R. P. Silver.** 1983. Six widespread bacterial clones among *Escherichia coli* K1 isolates. Infect. Immun. **39**:315–335.
3. **Achtman, M., and G. Pluschke.** 1986. Clonal analysis of descent and virulence among selected *Escherichia coli*. Annu. Rev. Microbiol. **40**:185–210.
4. **Caugant, D. A., B. R. Levin, and R. K. Selander.** 1981. Genetic diversity and temporal variation in the *Escherichia coli* population of a human host. Genetics **98**:467–490.
5. **Caugant, D. A., B. R. Levin, and R. K. Selander.** 1984. Distribution of multilocus genotypes of *Escherichia coli* within and between host families. J. Hyg. **92**:377–384.
6. **Cooke, E. M., S. Ervins, and R. A. Shooter.** 1969. Changing faecal populations of *Escherichia coli* in hospital medical patients. Br. Med. J. **4**:593–595.
7. **Daifuku, R., and W. E. Stamm.** 1986. Bacterial adherence to bladder uroepithelial cells in catheter-associated urinary tract infection. N. Engl. J. Med. **314**:1208–1213.
8. **Edwards, P. R., and W. H. Ewing.** 1972. The genus *Escherichia*, p. 48–68. *In* P. R. Edwards and W. H. Ewing (ed.), Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Company, Minneapolis.
9. **Engler, J. A., and M. P. van Bree.** 1981. The nucleotide sequence and protein-coding capability of the transposable element IS5. Gene **14**:155–163.
10. **Felmlee, T., S. Pellett, and R. A. Welch.** 1985. Nucleotide sequence of an *Escherichia coli* chromosomal hemolysin. J. Bacteriol. **163**:94–105.
11. **Fowler, J. E., and T. A. Stamey.** 1977. Studies of introital colonization in women with recurrent urinary tract infections. VII. The role of bacterial adherence. J. Urol. **117**:472–476.
12. **Green, L., R. D. Miller, D. E. Dykhuizen, and D. L. Hartl.** 1984. Distribution of DNA insertion element IS5 in natural isolates of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81**:4500–4504.
13. **Gross, R. J., and B. Rowe.** 1985. Serotyping of *Escherichia coli*, p. 345–364. *In* M. Sussman (ed.), The virulence of *Escherichia coli*. Academic Press, Inc., New York.
14. **Grunstein, M., and D. Hogness.** 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. USA **72**:3961–3965.
15. **Hagberg, L., R. Hull, S. Hull, S. Falkow, R. Freter, and C. Svanborg Edén.** 1983. Contribution of adhesion and bacterial persistence in the mouse urinary tract. Infect. Immun. **40**:265–272.
16. **Hanson, M. S., and C. C. Brinton.** 1988. Identification and characterization of *E. coli* type-1 pilus tip adhesion protein. Nature (London) **332**:265–268.
17. **Hartl, D. L., and D. E. Dykhuizen.** 1984. The population genetics of *Escherichia coli*. Annu. Rev. Genet. **18**:31–68.
18. **Hull, R. A., R. E. Gill, B. Hsu, B. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect. Immun. **33**:933–938.
19. **John, J. F., and J. A. Twitty.** 1986. Plasmids as epidemiological markers of gram-negative bacilli: experience at a university and review of the literature. Rev. Infect. Dis. **8**:693–704.
20. **Knapp, S., J. Hacker, I. Then, D. Muller, and W. Goebel.** 1984. Multiple copies of hemolysin genes and associated sequences in the chromosomes of uropathogenic *Escherichia coli* strains. J. Bacteriol. **159**:1027–1033.
21. **Korhonen, T. K., V. Vaisanen, H. Saxen, H. Hultberg, and S. B. Svenson.** 1981. P-antigen-recognizing fimbriae from human uropathogenic *Escherichia coli* strains. Infect. Immun. **37**:286–291.
22. **Korhonen, T. K., V. Vaisanen-Rhen, M. Rhe, A. Pere, J. Parkkinen, and J. Finne.** 1984. *Escherichia coli* fimbriae recognizing sialyl galactosides. J. Bacteriol. **159**:762–766.
23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
24. **Ling, G. V.** 1976. Antepubic cystocentesis in the dog: an aseptic technique for routine collection of urine. Calif. Vet. **30**:50–58.
25. **Ling, G. V., E. L. Bibestein, and D. C. Hirsh.** 1979. Bacterial

pathogens associated with urinary tract infections. Vet. Clin. North Am. Small Anim. Pract. **9**:617–630.

26. **Low, D., V. David, D. Lark, G. Schoolnik, and S. Falkow.** 1984. Gene clusters governing the production of hemolysin and mannose-resistant hemagglutination are closely linked in *Escherichia coli* serotype O4 and O6 isolates from urinary infections. Infect. Immun. **43**:353–358.
27. **Low, D., E. N. Robinson, Z. A. McGee, and S. Falkow.** 1987. The frequency of expression of pyelonephritis-associated pili is under regulatory control. Mol. Microbiol. **1**:335–346.
28. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Moch, T., H. Hoschutzky, J. Hacker, K.-D. Kroncke, and K. Jann.** 1987. Isolation and characterization of the α-sialyl-β-2,3-galactosyl-specific adhesin from fimbriated *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:3462–3466.
31. **Muller, D., C. Hughes, and W. Goebel.** 1983. Relationship between plasmid and chromosomal hemolysis determinants of *Escherichia coli*. J. Bacteriol. **153**:846–851.
32. **Norgren, M., S. Normark, D. Lark, P. O'Hanley, G. Schoolnik, S. Falkow, C. Svanborg-Eden, M. Baga, and B.-E. Uhlin.** 1984. Mutations in *Escherichia coli* cistrons affecting adhesion to human cells do not abolish *pap* pili fiber formation. EMBO J. **3**: 1159–1165.
33. **Normark, S., D. Lark, R. Hull, M. Norgren, M. Baga, P. O'Hanley, G. Schoolnik, and S. Falkow.** 1983. Genetics of digalactoside binding adhesin from a uropathogenic *Escherichia coli* strain. Infect. Immun. **41**:942–949.
34. **O'Hanley, P., D. Lark, S. Falkow, and G. Schoolnik.** 1985. Molecular basis of *Escherichia coli* colonization of the upper urinary tract in BALB/c mice: Gal-Gal pili immunization prevents *E. coli* pyelonephritis in the BALB/c mouse model of human pyelonephritis. J. Clin. Invest. **75**:347–360.
35. **O'Hanley, P., D. Lark, S. Normark, S. Falkow, and G. Schoolnik.** 1983. Mannose-sensitive and gal-gal binding *Escherichia coli* pili from recombinant strains: chemical functional and serological properties. J. Exp. Med. **158**:1713–1719.
36. **O'Hanley, P., D. Low, I. Romero, D. Lark, K. Vosti, S. Falkow, and G. Schoolnik.** 1985. Gal-gal binding and hemolysin phenotypes and genotypes associated with uropathogenic *Escherichia coli*. N. Engl. J. Med. **313**:414–420.
37. **Orskov, I., F. Orskov, A. Brich-Anderson, M. Kanamori, and C. Svanborg-Eden.** 1982. O, K, H and fimbrial antigens in *Escherichia coli* serotypes associated with pyelonephritis and cystitis. Scand. J. Infect. Dis. Suppl. **33**:18–25.
38. **Portnoy, D. A., and S. Falkow.** 1981. Virulence-associated plasmids from *Yersinia enterocolitica* and *Yersinia pestis*. J. Bacteriol. **148**:877–883.
39. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to a high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
40. **Roberts, A. P., J. D. Linton, M. M. Waterman, P. E. Gower, and K. G. Koutsaimanis.** 1975. Urinary and faecal *Escherichia coli* O-serotypes in symptomatic urinary tract infection and asymptomatic bacteriuria. J. Med. Microbiol. **8**:311–318.
41. **Seed, B.** 1982. Diazotizable arylamine cellulose papers for coupling and hybridization of nucleic acids. Nucleic Acids Res. **10**:1799–1810.
42. **Smith, B. J.** 1984. SDS polyacrylamide gel electrophoresis of proteins, p. 41–56. *In* J. M. Walker (ed.), Methods in molecular biology, vol. 1. Proteins. The Humana Press, Clifton, N.J.
43. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
44. **Stamey, T. A., M. Timothy, M. Millar, and G. Mihara.** 1971. Recurrent urinary infections in adult women: the role of introital bacteria. Calif. Med. **115**:1–19.
45. **Vaisanen, V., T. K. Korhonen, M. Jokinen, C. G. Gahmberg, and C. Ehnholm.** 1982. Blood group M specific hemagglutinin in pyelonephritogenic *Escherichia coli*. Lancet **i**:1192.
46. **Vosti, K. L., L. M. Goldberg, A. S. Momto, and L. A. Rantz.** 1964. Host-parasite interaction in patients with infections due to *Escherichia coli*. I. The serogrouping of *E. coli* from intestinal and extraintestinal sources. J. Clin. Invest. **43**:2377–2385.
47. **Welch, R. A., E. P. Dellinger, B. Minshew, and S. Falkow.** 1981. Haemolysin contributes to virulence of extraintestinal *Escherichia coli* infections. Nature (London) **294**:665–667.
48. **Welch, R. A., R. Hull, and S. Falkow.** 1983. Molecular cloning and physical characterization of a chromosomal hemolysin from *Escherichia coli*. Infect. Immun. **42**:178–186.
49. **Westerlund, B., A. Pere, T. K. Korhonen, A.-K. Jarvinen, A. Siitonen, and P. H. Williams.** 1987. Characterization of *Escherichia coli* strains associated with canine urinary tract infections. Res. Vet. Sci. **42**:404–406.