Expression of Type 1 Fimbriae May Be Required for Persistence of *Escherichia coli* in the Catheterized Urinary Tract

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Long-term urinary catheterization results in polymicrobial bacteriuria and is complicated by fever, bacteremia, acute pyelonephritis, and death. *Escherichia coli* is a common urine isolate from catheterized patients and can persist for months. We hypothesized that fimbria-mediated adherence contributes to its persistence. For 1 year, urine specimens were collected from 51 patients ≥ 65 years of age who were catheterized for ≥ 30 days. *E. coli* was isolated at $\geq 10^5$ CFU/ml from 447 (36%) of 1,230 weekly urine specimens from 26 patients. Week 1 isolates from 52 definable episodes were tested for hemagglutination, hybridization with gene sequences from the *pil* and *pap* operons, in vitro adherence to catheter material, binding of ¹²⁵I-labeled Tamm-Horsfall protein, hemolysin and colicin V production, and serum resistance. The proportions of isolates of short (1 week only), medium (2 to 11 weeks) and long (≥ 12 weeks) episodes of bacteriuria which expressed type 1 fimbriae as assayed by mannose-sensitive hemagglutination were 59, 65, and 92%, respectively. Isolates with the *pil* operon (the genome for type 1 fimbriae) from episodes lasting >1 week expressed mannose-sensitive hemagglutination more frequently ($P = 0.011$) than *pil*-positive isolates from episodes of ≤ 1 week. Isolates from episodes of >1 week also bound significantly more Tamm-Horsfall protein than isolates from episodes of ≤ 1 week ($P = 0.044$). Although nearly half of the isolates produced P fimbriae, an important virulence factor for the development of pyelonephritis, no correlation with persistence could be made. Overall, the *E. coli* isolates expressed traits similar to those of strains that cause cystitis. Type 1 fimbriae appear to be important for the persistence of *E. coli* in the long-term-catheterized urinary tract.

Bacteriuria is commonly associated with indwelling urinary catheterization (37, 38). Once a urethral catheter is in place, the risk of bacteriuria is 5 to 10%/day (38). Therefore, virtually all patients with catheters in place for weeks and months are bacteriuric (38). Estimates of the number of nursing home patients (1.3 million in 1977) (13) and the prevalence of long-term catheterization (9% of patients) (13) suggest that catheter-associated bacteriuria is the most common nosocomial infection in the United States. While usually asymptomatic, such patients can experience fever, acute pyelonephritis, bacteremia, and death (37a).

Prospective microbiologic studies of these patients have revealed dynamic, polymicrobial bacteriuria. Commonly isolated organisms include *Providencia stuartii*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and enterococci (38). While the incidences of new episodes of bacteriuria by these species are quite similar, the duration of episodes, i.e., the ability to persist in the catheterized urinary tract, varies considerably among these species. However, two organisms, *E. coli* and *P. stuartii*, persisted significantly longer than all other species, for weeks and months (38). The mechanisms of this persistence have not been well described.

We hypothesized that the presence of a long-term urinary catheter forms an abnormal urinary tract, thus reducing the need for expression of P fimbriae (4, 9-12, 14, 21, 24, 25, 30, 33, 35) and possibly other virulence factors, including hemolysin (8, 26, 30); serum resistance (3); colicin production (26, 32); certain O, K, or H serotypes (4); and the ability to

ferment sucrose and salicin (4). We report here that *E. coli* isolates that are able to persist in the catheterized urinary tract may depend on expression of type 1 fimbriae and may not require P fimbriae or other virulence factors for persistent colonization and pathogenesis.

(A preliminary account of this work has been presented [H. L. T. Mobley, G. R. Chippendale, J. H. Tenney, and J. W. Warren, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, D118, p. 85].)

MATERIALS AND METHODS

Patients and specimens. Fifty-one patients 65 years or older with urinary catheters in place for ≥ 30 days resided at two chronic-care facilities in Baltimore, Md. For 1 year, weekly urine specimens were aseptically collected by needle aspiration from the catheter and transported on ice within 4 h to the Division of Infectious Diseases microbiology laboratory, University of Maryland School of Medicine.

Bacterial identification. Urine, diluted 1:100 in sterile saline (0.85% [wt/vol] NaCl), was inoculated by a calibrated 0.001-ml loop onto eosin-methylene blue agar and Trypticase soy agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.). Gram-negative bacilli from pure cultures were identified to species by the Minitek Enterobacteriaceae II System (BBL). Isolates were stored at -70°C in Trypticase soy broth supplemented with 15% (vol/vol) glycerol. O serotypes were determined by the *E. coli* Reference Center, Pennsylvania State University, University Park.

Episodes of bacteriuria. Episodes of bacteriuria were defined as previously described (38). Briefly, the beginning of

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an episode was defined by the isolation of an organism that was not identified in two previous weekly specimens. The end of an episode was defined by failure to identify the organism for two consecutive cultures. Within an episode, the biotypes of the organisms, as defined by the Minitek numerical taxonomy code, could not differ from week to week by more than two biochemical reactions. Week 1 isolates of each bacteriuric episode were used in all assays. Episodes were arbitrarily defined as short (isolated from one weekly specimen), medium (2 to 11 weeks), and long (lasting ≥ 12 weeks).

HA assay. *E. coli* isolates were grown at 37°C overnight on Trypticase soy agar for assay of mannose-resistant hemagglutination (MRHA) and statically in Luria broth for 48 h, 37°C, for assay of mannose-sensitive hemagglutination (MSHA). A suspension of 1% (vol/vol) human type O erythrocytes supplemented with 50 mM mannose was used for MRHA; a suspension of 1% (vol/vol) guinea pig erythrocytes was tested for MSHA in the presence and absence of 50 mM mannose. All HAs were assayed on rocked ceramic tiles at 4°C.

For HA inhibition by Tamm-Horsfall protein (THP), broth-grown bacteria were suspended to a concentration of about 10^9 CFU/ml in 50 mM sodium phosphate–145 mM sodium chloride (pH 7.2; PBS). The suspension (1 ml) was incubated with solubilized THP (1 mg/ml) (see below) for 30 min at 37°C and harvested by centrifugation, and the supernatant was aspirated. The cell pellet was suspended in 1 ml of PBS. MSHAs with twofold dilutions of bacterial suspensions incubated with THP were compared with those of dilutions of suspensions incubated without THP.

Hemolysin production. *E. coli* isolates were tested for beta-hemolysis by inoculation onto 5% sheep blood agar plates. Hemolysis was defined by a distinct zone of clearing around isolated bacterial colonies after overnight incubation at 37°C. All assays were done in duplicate.

Serum resistance assay. Bacterial suspensions were adjusted to the turbidity of a McFarland no. 1 standard (approximately 3×10^8 CFU/ml), and 17- μ l samples were mixed with 1 ml of 40% pooled human serum in sterile PBS or sterile PBS (control) and incubated for 60 min at 37°C (3). Each tube was diluted 1,000-fold and then 0.1 ml was inoculated onto spread plates. After overnight incubation, all colonies were counted. Sensitivity was defined as a 90% or greater reduction in CFU of cells incubated with serum compared with the PBS control (3).

Colicin V production. Bacterial isolates grown for 24 h on 5% sheep blood agar plates were streaked onto nutrient agar plates. After overnight incubation at 37°C, the agar surfaces were exposed to chloroform vapor for 30 min and then to air for 30 min. Each plate was overlaid with 5 ml of nutrient agar (1.5% agar) containing 0.25 ml of *E. coli* indicator strain HB101 or C600 (10^9 CFU/ml) and incubated overnight at 37°C (29, 32). Colicin V production was defined by a zone of clearing around bacterial isolates in the overlay of C600 but not of HB101 (32).

Dot blot hybridization. Bacterial cultures were lysed and denatured in alkaline sodium dodecyl sulfate, spotted onto nitrocellulose, neutralized, dried, and baked, as described by Maniatis et al. (27). DNA fragments were isolated by electroelution of restricted plasmids from an agarose gel; extracted with phenol, chloroform, and ether; and labeled with [32 P]ATP by nick translation (27). The *pap* fimbrial probe represents the *Hind*III C fragment of pRHU845 described by Hull et al. (15). The *pil* fimbrial probe represents the *Acc*I fragment within the *Sal*I fragment of pSH2 de-

scribed by Buchanan et al. (5). Hybridizations were performed as previously described (5). *E. coli* SH1 (*pap*⁺ *pil*⁺) was used as a positive control.

In vitro catheter adherence assay. Catheter adherence was assayed by modifications to the method of Sugarman (36). Bacterial strains were grown statically for 48 h in 4 ml of minimal salts medium (28) supplemented with 0.5% (wt/vol) glycerol containing 5 μ Ci of [3 H]thymidine (75.5 Ci/mmol; New England Nuclear Corp., Boston, Mass.); the cells were harvested by centrifugation and suspended in PBS (pH 7.2) to a concentration of 5×10^8 CFU/ml. Bacterial suspension (1.5 ml) was added to a culture tube (12 by 75 mm) containing a 2.0-cm section (surface area, 580 mm²) of siliconized latex catheter (18 French, 5 cm³; Travenol Laboratories, Deerfield, Ill.). Tubes were incubated for 90 min at 37°C. The catheter segment was retrieved with forceps, placed in a 20-ml scintillation vial containing 10 ml of PBS (pH 7.2), rinsed with two additional changes of PBS, and incubated for 15 min in 2 ml of 0.1 M NaOH. After incubation, the solution was neutralized with 0.2 ml of 1 M HCl. Radioactivity was determined by scintillation counting after the addition of Aqueous Counting Scintillant (Amersham Corp., Arlington Heights, Ill.) to each vial. Counts per minute were normalized to adherent bacteria per square millimeter of catheter material.

THP binding assay. THP was purified from 10 liters of pooled human urine by repeated precipitation in 0.58 M NaCl as described by Schachner et al. (34). Homogeneity was determined by identifying a single Coomassie blue-stained band on an overloaded sodium dodecyl sulfate-10% polyacrylamide gel (23). THP (40 μ l; 1 mg/ml) in 10 mM sodium phosphate (pH 7.2) was labeled with 0.5 mCi of 125 I (carrier-free; New England Nuclear Corp.) in the presence of chloramine T by modifications to the method of Hunter and Greenwood (17) as described by Schachner et al. (34). Iodinated protein was desalted on a Sephadex G-25 column (20 by 1 cm) using distilled water for elution. Fractions containing radiolabeled protein were stored in lead containers at 4°C and used within 4 days. Before use, 125 I-labeled THP was diluted in 10 mM phosphate buffer containing 1% crystalline bovine serum albumin.

Binding of THP by bacteria was determined by a modification of the method of Schachner et al. (34) as follows. Bacteria were grown statically in nutrient broth for 48 h, harvested by centrifugation, and suspended in 5 mM sodium phosphate (pH 7.2) to an optical density of 0.5 at 550 nm (1-cm path length). Suspensions (1 ml) were added to 1.5-ml microcentrifuge tubes along with 25 to 35 μ l of 125 I-labeled THP (100,000 cpm) and incubated at 23°C for 60 min. Samples (450 μ l) were pipetted into each of two tubes. Bacteria were sedimented in a microcentrifuge (Eppendorf) at maximum speed ($15,600 \times g$) for 1 min. The supernatant was aspirated, and the cell pellet was suspended in 1 ml of 5 mM phosphate buffer (pH 7.2). The cells were again sedimented, and the supernatant was discarded. Radioactivity in tubes containing washed cell pellets was determined by gamma spectroscopy. Total precipitable 125 I-labeled THP was determined in the absence of bacteria by simultaneous incubations with cold 10% (wt/vol) trichloroacetic acid in the presence of bovine serum albumin (100 μ g/ml). Phytohemagglutinin (200 μ g/ml), a mannose-specific lectin, was used as a control for precipitation by mannose-binding receptors. Tubes containing labeled THP in 5 mM phosphate buffer were used to determine background counts. Assays were performed in duplicate, and values were reproducible within 1%.

TABLE 1. Defined episodes of *E. coli* catheter-associated bacteriuria

Length of episode (definition)	No. of episodes	No. of patients	No. of distinct O serotypes	No. of episodes caused by O serotype:						
				75	1	6	3	83	8	7
Short (1 wk)	22	11	8	2	5	2	3	1	0	0
Medium (2–11 wk)	17	14	10	3	1	1	0	2	1	2
Long (≥ 12 wk)	13	11	8	1	0	2	1	1	2	0
All	52	26	14							

RESULTS

Episodes of bacteriuria. *E. coli* strains were isolated at a concentration of $\geq 10^5$ CFU/ml from 447 (36%) of 1,230 urine specimens from long-term-catheterized patients. The strains could be grouped into 52 defined episodes of bacteriuria from 26 patients (Table 1). Of 52 episodes, 22 (42%) lasted for 1 week or less, whereas the remaining 30 episodes ranged in duration from 2 to 41 weeks, with 13 episodes lasting 12 or more weeks.

Of 52 first isolates, 39 were O typeable and represented 14 distinct serotypes, thus indicating multiple strains and not the spread of a single nosocomial organism. Of the 13 nontypeable isolates, 2 were rough and 11 were smooth. More than half of the episodes (28 of 52) were caused by isolates with six O serotypes. Nineteen isolates were serotype O1, O6, O7, or O75, serotypes which have been found to cause a high proportion of urinary tract infections (4). Those 19 isolates represented 49% of the 39 typeable isolates and 37% of the total 52 isolates.

MSHA and *pil* genotype. The proportions of first isolates of short, medium, and long episodes that expressed MSHA were 59, 65, and 92%, respectively (Table 2). Long-episode representatives expressed MSHA significantly more often (12 of 13; 92%) than isolates from short or medium episodes (24 of 39; 62%) ($P = 0.035$). The 36 isolates expressing MSHA had a mean episode duration of 10.3 ± 12.7 (standard deviation) weeks, which was significantly longer than that of the 16 isolates not expressing MSHA (2.9 ± 3.8 weeks) ($P = 0.022$).

Because of this association, the expression of type 1 fimbriae was examined more closely (Table 3). Organisms isolated for a single week only (short episodes) were compared with organisms isolated from episodes of 2 weeks or more. Although about the same percentage of organisms from the two groups possessed the *pil* genome (i.e., hybridized with *pil* gene sequences), isolates of this genotype from longer episodes of bacteriuria (>1 week) expressed MSHA at a significantly higher frequency ($P = 0.011$) than *pil*-positive isolates from short episodes (≤ 1 week).

MRHA and *pap* genotype. The results (Table 2) indicated

no correlation of phenotypic or genotypic presence of P fimbriae with persistence of *E. coli* in the catheterized urinary tract. Similarly, there was no significant difference between the mean durations of bacteriuric episodes between isolates expressing MRHA (7.1 ± 11.5 weeks) and those not expressing MRHA (8.7 ± 11.3 weeks).

Other virulence factors. Few organisms expressed hemolysin as assayed by phenotypic expression on blood agar (7 of 52; 13%). Colicin V was detected in 14 of 52 (27%) isolates. No correlation of hemolysin or colicin V production with persistence could be made. Most of the isolates were resistant to killing by pooled normal human sera. Isolates from short episodes (21 of 22; 95%) tended to be more resistant than isolates from long episodes (9 of 13; 69%).

THP binding. Bacterial suspensions of first isolates were incubated with 125 I-labeled THP. Cells were washed, and cell-bound THP was measured. The mean binding for *E. coli* isolates (5×10^8 bacteria per ml) was $2,380 \pm 4,130$ cpm of 125 I-labeled THP ($n = 51$) compared with a mean of 29,700 cpm for phytohemagglutinin (control for precipitation by mannose receptors) and 39,300 cpm for trichoroacetic acid (control for total protein-bound 125 I).

When binding data were organized according to length of episode, isolates from episodes lasting 2 weeks or more (medium and long) bound significantly more THP ($3,390 \pm 5,220$ cpm; $n = 29$) than isolates from short episodes ($1,030 \pm 590$ cpm; $n = 22$) ($P = 0.044$). The 35 isolates expressing MSHA tended to bind more THP ($2,600 \pm 4,480$ cpm) than the 16 isolates not expressing this hemagglutinin ($1,031 \pm 585$); however, this difference was not significant ($P > 0.1$).

Because of the suggestion that strains exhibiting MSHA could bind THP, HA inhibition studies were done.

Preincubation of *E. coli* HU9687, an MSHA⁺ strain, with THP inhibited MSHA. MSHA⁺ bacteria hemagglutinated guinea pig erythrocytes in the presence of 50 mM mannose at a dilution of 1:16. Suspensions preincubated with THP hemagglutinated at a dilution of 1:4.

In vitro catheter adherence. First isolates of bacteriuric episodes were labeled with tritiated thymidine and tested for in vitro adherence to standardized catheter segments. Values for 50 isolates ranged from 2,300 to 120,300 adherent

TABLE 2. Phenotypic and genotypic traits of first isolates from defined episodes of *E. coli* catheter-associated bacteriuria

Length of episode	No. of first isolates	No. (%) of isolates			
		MSHA ^a	<i>pil</i> ⁺ ^b	MRHA ^c	<i>pap</i> ⁺ ^d
Short	22	13 (59)	20 (91)	14 (64)	14 (64)
Medium	17	11 (65)	11 (65)	3 (18)	8 (47)
Long	13	12 (92)	13 (100)	6 (46)	9 (69)

^a HA of guinea pig erythrocytes, inhibitable by 50 mM mannose.

^b Positive dot blot hybridization with *pil* gene probe.

^c HA of human type O erythrocytes in the presence of 50 mM mannose.

^d Positive dot blot hybridization with *pap* gene probe.

TABLE 3. Expression of type 1 fimbriae in *pil*⁺ isolates

Length of episode	No. of isolates <i>pil</i> ⁺ by DNA hybridization ^a /no. of first isolates (%)	No. of isolates expressing MSHA ^b /no. of <i>pil</i> ⁺ isolates (%)
≤ 1 wk ^c	20/22 (91)	13/20 (65) ^d
> 1 wk ^e	24/30 (80)	23/24 (96)

^a Dot blot hybridization with *pilA* gene sequences.

^b Agglutination of 3% guinea pig erythrocyte suspension; inhibited by 50 mM mannose.

^c Isolated from one weekly specimen only.

^d $P = 0.011$ by Fisher exact test.

^e Isolated from two or more consecutive weekly urine specimens.

bacteria per mm² of catheter surface. The mean values for isolates from short (28,500 $\pm$ 29,700), medium (24,800 $\pm$ 15,700), and long (30,000 $\pm$ 19,500) episodes did not differ significantly ($P > 0.1$). The adherence of isolates expressing MSHAs (29,300 $\pm$ 26,200) did not differ from those not expressing MSHA (24,000 $\pm$ 16,000) ($P > 0.1$).

DISCUSSION

Our data indicate that expression of type 1 fimbriae may contribute to the ability of *E. coli* to persist in the catheterized urinary tract. Several reports suggest that expression of type 1 fimbriae is important in bladder colonization in animal models of infection (2, 6, 16, 18, 20). Bladder colonization by type 1-deficient strains of *E. coli* generated by nitrosoguanidine mutagenesis (18) or selection of non-fimbriate phase variants (16) or mutants specifically deleted for sections of the *pil* operon (20) was significantly reduced compared with that of the parental strains. These data are in agreement with our observation that, from all *pil*-positive isolates, first isolates of episodes lasting 1 week or less expressed type 1 fimbriae much less frequently (65%) than first isolates from longer episodes (96%). We postulate that type 1 fimbriate cells either colonize the bladder or adhere to THP that coats uroepithelial cells or the indwelling catheter surface.

Most *E. coli* isolates have the ability to synthesize type 1 fimbriae. The genes encoding these structures are highly conserved and reside on a 24-kilobase *Hind*III fragment in most isolates (5). The structural gene for the fimbriae is separable from the gene encoding the polypeptide with mannoside-binding specificity (20). Transcription of the genes for the *fim* operon, which encodes a similar type 1 fimbria, is turned on and off by inversion of a 314-base-pair DNA segment which inactivates the promoter (1, 7). This reversible switching occurs at a rate of 10⁻³ per cell per generation, results in expression or lack of expression of type 1 fimbriae, and is termed phase variation. Several investigators have postulated that phase variation per se may be a virulence factor (7, 16). That is, the same specificity for mannosides that allows initial colonization to bladder mucosa may also result in entrapment and elimination of the organism by Tamm-Horsfall urinary glycoprotein (31) or polymorphonuclear leukocytes (22).

The expression of P fimbriae was not correlated with the ability of *E. coli* to persist in the catheterized urinary tract. This fimbria has been established as a critical virulence factor in *E. coli* isolates causing acute pyelonephritis in patients free of urinary tract abnormalities (25). The adhesin component (24) of this fimbria specifically recognizes a digalactoside moiety of the P blood group glycolipid of the globoseries which is present on uroepithelial cells in the urinary tract lining (26) and in the kidney parenchyma (21). Wild-type isolates possessing P fimbriae or fecal isolates harboring recombinant plasmids encoding P fimbriae are more likely to cause acute pyelonephritis in a mouse model

of unobstructed ascending urinary tract infection than isolates lacking these structures (10, 11). However, in patients with anatomic or physiologic abnormalities of the urinary tract, P fimbriae were found not to be essential for the establishment of kidney infection (25). The catheterized urinary tract may present an analogous situation in which the presence of a foreign body in the bladder or the obstruction of urine flow in catheters blocked by struvite deposits may result in obstruction or reflux, thus obviating the need for P fimbria expression.

Adherence to catheter material per se also did not appear to be a mechanism of persistence for *E. coli*. This is not surprising, since *E. coli* is a common cause of urinary tract infection in both noncatheterized and catheterized patients. This is in sharp contrast to infection with *P. stuartii*, a bacterial species associated with persistent catheter-associated bacteriuria but rarely seen in the noncatheterized patient. *P. stuartii* isolates causing persistent infection bind in higher numbers in vitro to catheter surfaces compared with *E. coli* (H. L. T. Mobley, G. R. Chippendale, J. H. Tenney, A. R. Mayrer, L. J. Crisp, J. L. Penner, and J. W. Warren, J. Infect. Dis., in press). In addition, duration of bacteriuric episodes for *P. stuartii* isolates was correlated neither with expression of type 1 fimbriae nor with THP binding as we have demonstrated for *E. coli*. Rather, *P. stuartii* catheter adherence correlates with the MR/K hemagglutinin, an adhesin not expressed by *E. coli*.

The relative importance of potential virulence factors has been examined epidemiologically by determining the presence or absence of the factor in strains isolated from patients with pyelonephritis, cystitis, or asymptomatic bacteriuria (4, 9, 12, 19, 26, 30) (Table 4). As a group, isolates from catheterized patients do not share the profile of the highly virulent organisms associated with pyelonephritis. The *E. coli* isolates described in this report expressed P fimbriae and colicin V in proportions similar to those of strains isolated from cases of cystitis. In addition, type 1 fimbriae were expressed by 79% (26 of 33) of the persistent (>1-week) isolates, comparable to pyelonephritogenic strains. On the other hand, hemolysin production in isolates from catheter-associated bacteriuria was very limited (13% of isolates), similar to the occurrence in fecal isolates. Many of these *E. coli* isolates possess the virulence factors associated with symptomatic bladder infection and do not simply represent random colonization by avirulent fecal organisms. We speculate that in the presence of a long-term indwelling catheter these traits are sufficient for persistent colonization and pathogenesis.

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TABLE 4. Comparison of frequencies of virulence factors in *E. coli* from catheter-associated bacteriuria and other clinical sources

Source of isolates	No. of isolates/total (%)				Reference(s)
	MSHA	MRHA	Hemolysin	Colicin V	
Pyelonephritis	98/123 (80)	134/172 (78)	31/50 (62)	14/31 (45)	4, 12, 19, 26, 30
Cystitis	117/129 (90)	66/167 (40)	83/169 (49)	19/64 (30)	4, 12, 19, 26, 30
Asymptomatic bacteriuria	75/125 (60)	19/132 (14)	11/42 (26)	3/13 (23)	4, 12, 19, 26, 30
Feces	137/193 (71)	44/314 (14)	47/268 (18)	5/39 (13)	4, 12, 19, 26, 30
Catheter-associated bacteriuria	36/52 (69)	23/52 (44)	7/52 (13)	14/52 (27)	This work

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