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Quantitation of Effects of Subinhibitory Concentrations of Trimethoprim on P Fimbria Expression and In Vitro Adhesiveness of Uropathogenic *Escherichia coli*

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The ability to adhere to and colonize urogenital mucosa is an important virulence attribute of uropathogenic *Escherichia coli*. This adherence, which appears to be mediated by P fimbriae, may be affected by antibiotics or other agents that affect fimbrial expression. We describe here an enzyme immunofiltration assay to quantitate fimbriation and the application of that technique to measurement of the effects of sublethal doses of trimethoprim on P fimbrial expression. Effects on P fimbriation correlated with effects on the adherence of treated bacteria to cultured T24 bladder carcinoma epithelial cells; i.e., trimethoprim treatment decreased both P fimbriation and bacterial adherence. It was possible to quantitate effects on P fimbriation when type 1 fimbriae were also present. The enzyme immunofiltration assay may be useful for studies on the role of fimbriae in the pathogenesis of bacterial infections, and it may facilitate identification of antimicrobial agents that interfere with bacterial adherence to mucosal surfaces.

Escherichia coli is the microorganism most commonly isolated from endogenous human urinary tract infections. The ability to adhere to uroepithelial cells and colonize urogenital mucosa is an important virulence attribute of uropathogenic strains of *E. coli* (UPEC) (16, 26). The presence of P fimbriae on UPEC correlates with their ability to adhere to uroepithelial cells in vitro (15, 16, 26, 27). The P fimbriae (P pili, pap pili, digalactoside-binding pili, Gal-Gal pili) are proteinaceous, filamentous appendages with the ability to agglutinate human P blood group erythrocytes in the presence of mannose (mannose-resistant hemagglutination; 9, 13, 15, 16, 31, 33).

The role of fimbria-mediated adherence of enterotoxigenic *E. coli* to intestinal mucosa in the pathogenesis of enterotoxigenic *E. coli* diarrheal disease has been demonstrated (7). By analogy, P fimbriae are believed to mediate the adherence of UPEC to urogenital mucosa and to facilitate colonization and invasion of the urogenital mucosa by these bacteria.

Interference with the ability of UPEC to express P fimbriae might interfere with their ability to adhere to uroepithelial mucosa and aid in the prophylaxis or the treatment (or both) of urinary tract infections. Several investigators have reported that sub-MICs of some antibiotics decrease the ability of UPEC to adhere to uroepithelial cells (24, 25, 28), buccal cells (21, 32), cultured human intestinal cells (32), and human erythrocytes (14, 32, 34) in vitro. Decreased in vitro bacterial adhesiveness appeared to correlate with decreased fimbriation as determined by mannose-resistant hemagglutination (32) or electron microscopy (21, 28, 32). Neither mannose-resistant hemagglutination nor the characteristic appearance seen by electron microscopy are specific for P fimbriae. A method specific for P fimbriae is needed to identify antimicrobial agents that affect P fimbria expression and may have antiadhesive properties.

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We describe here a quantitative enzyme immunofiltration (EIF) assay for detection of cell-bound P fimbriae. Using the EIF assay to quantitate the effects of sub-MIC trimethoprim treatment on the expression of P fimbriae by UPEC, we showed that effects on P fimbriae correlate with effects on the ability of UPEC to adhere to cultured human bladder (T24) cells. The specificity of the EIF assay allows quantitation of P fimbriae in the presence of type 1 fimbriae (mannose-sensitive hemagglutinins) that are commonly found on UPEC but have no apparent role in urogenital colonization (15, 20).

MATERIALS AND METHODS

Bacterial strains and culture conditions. The *E. coli* strains used in this study are listed in Table 1. These derivatives of *E. coli* J96 (O4, K6), an isolate from a patient with acute pyelonephritis (20), were generously provided by Sheila Hull (Baylor College of Medicine, Houston, Tex.). *E. coli* SH1 *lac* is motile, hemolytic, positive for colicin V, serum resistant, and capable of expressing both P (digalactoside-binding) fimbriae and type 1 (mannose-binding) fimbriae (11). Strain SH1-P is a variant of SH1 that expresses P fimbriae, but not type 1 fimbriae, when cultured on tryptic soy agar (GIBCO Laboratories, Grand Island, N.Y.), blood agar (tryptic soy agar with 5% sheep blood), or in shaken tryptic soy broth (TSB; GIBCO) as described below. Strain SH1-P bacteria express both P and type 1 fimbriae when grown statically in TSB. *E. coli* HU849 and SH48 are derivatives of nonfimbriate *E. coli* K-12 P678-54, which contains recombinant plasmids prepared from *E. coli* J96, the gene sequences necessary for the expression of P (pRHU845) or type 1 (pSH2) fimbriae, respectively (10).

All cultures were maintained and grown under conditions that promoted P fimbria expression (vide infra). Stock cultures were maintained on tryptic soy agar or blood agar plates at 4°C. Cells from isolated colonies were tested for fimbriae by the slide agglutination (SA) method by using fimbria-specific antiserum (19) or by latex carbohydrate agglutination by using α-D-Gal-1,4β-D-Gal–O$(CH_2)_8COOCH_3$ (Syn Gal-Gal) adsorbed to latex beads (Chembiomed, Ed-

TABLE 1. Fimbria antigen detection and carbohydrate-binding properties of *E. coli* strains

Strain	Fimbria antigen[a]		Carbohydrate binding[b]		
	P	Type 1	Syn Gal-Gal	Syn Man-Man	Glucose
SH1 (J96; O4, K6)	+	+	+	+	−
SH1-P[c]	+	−	+	−	−
SH1, SH1-P (18°C)	−	−	−	−	−
P678-54 (host strain)	−	−	−	−	−
HU849 (P678-54 with pRHU845)[d]	+	−	+	−	−
SH48 (P678-54 with pSH2)[d]	−	+	−	+	−

[a] Fimbria antigen was detected by the SA method (19) using fimbria-specific antisera. +, Positive; −, negative.

[b] Agglutination of latex beads adsorbed with Syn Gal-Gal (P fimbria receptor analog), Syn Man-Man (type 1 fimbria receptor analog), or glucose (22) was measured.

[c] The strain was cultured in TSB and incubated at 37°C with shaking.

[d] This is a recombinant plasmid from *E. coli* J96.

monton, Alberta, Canada) for P fimbriae or α-D-Man-1,2α-D-Man–O(CH)$_8$COOCH$_3$ (Syn Man-Man) adsorbed to latex beads (Chembiomed) for type 1 fimbriae, as previously described by O'Hanley et al. (22). Fimbriate colonies were subcultured in TSB, and cultures were incubated at 37°C with shaking for the times specified. Cultures were incubated at 18°C to suppress fimbria expression (12). All cultures were tested for P and type 1 fimbriae by SA or latex carbohydrate agglutination before use in the assays.

Fimbria purification. P and type 1 fimbriae were purified from TSB cultures of *E. coli* HU849 and SH48, respectively, by the method of Brinton (1) as modified by Normark et al. (20). The purity of the fimbriae was demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. HU849 P fimbriae formed a single band, and SH48 type 1 fimbriae formed two bands characteristic of mannose-sensitive fimbriae, as previously described (22).

Antibody production. Antisera against P and type 1 fimbriae were produced in New Zealand White rabbits by three subcutaneous injections with purified P or type 1 fimbriae. Each injection consisted of 50 to 75 μg of fimbrial protein in phosphate-buffered saline (PBS) mixed with an equal volume of Freund complete adjuvant (first two injections) or without adjuvant. Injections were administered at 3- to 4-week intervals, and animals were bled by cardiac puncture 9 to 14 days after the final injection. Nonfimbrial antibodies were absorbed by using strain P678-54, the nonfimbriate K-12 parent strain, as described by Edwards and Ewing (5). The immunoglobulin G (IgG) fraction was prepared by using DEAE–Affi-gel Blue chromatography (Bio-Rad Laboratories, Richmond, Calif.), according to manufacturer instructions. Fimbrial specificity of the IgGs was demonstrated by SA, Western blots (immunoblots), and EIF.

Protein determinations. Protein concentrations were estimated by the method of Lowry et al. (18), with bovine serum albumin as the standard.

Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed in 15% polyacrylamide vertical slab gels by the method of Laemmli (17). Proteins were stained by Coomassie blue (6).

Western blot analysis. After separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, proteins were electroblotted onto nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, N.H.) as described by Towbin (29). Unreacted sites on the nitrocellulose were blocked by three 10-min washes in 0.05% Tween 20 (Sigma Chemical Co., St. Louis, Mo.) in PBS by the method of De Blas and Cherwinski (3). The filters were sequentially incubated in diluted rabbit antifimbrial IgG, washed, incubated in horseradish peroxidase-conjugated anti-rabbit immunoglobulins prepared in swine (Accurate Chemical Co., Westbury, N.Y.), washed, and then developed with horseradish peroxidase color development reagent (4-chloro-1-naphthol; Bio-Rad) prepared as directed by the manufacturer. All dilutions and washes were performed with 0.05% Tween 20 (Sigma) in PBS.

EIF assay. Cell-bound fimbriae on *E. coli* were quantitated by an EIF assay that was a modification of the enzyme-linked antibody centrifuge assay described by Isaacson (12). The EIF assay was performed by using the Microfold immunofiltration system (Isolab, Akron, Ohio), consisting of a disposable 96-well immunofiltration plate that rests on a vacuum manifold. This unit served as the incubation chamber and washing device for the subsequent immunoassay steps after bacteria were immobilized by filtration on glass fiber filters on the plates. In the EIF assay, washes were performed by vacuum filtration rather than by centrifugation as in the previously described enzyme-linked antibody centrifuge assay (12).

To prevent nonspecific binding to the filters or the plastic wells, all wells in the Microfold were filled with 3% bovine serum albumin in PBS and incubated at 37°C for 1 h. Equal volumes of bacteria (washed with PBS by centrifugation and resuspended in PBS to an A_{650} of 0.250) and antifimbrial IgG were added to the Microfold wells and incubated for 1 h at 22°C. Bacteria and bound antibody were then immobilized on the filters by filtration, and unbound antibody was washed away by three 250-μl washes with 3% bovine serum albumin in PBS. Next, alkaline phosphatase-conjugated anti-rabbit immunoglobulins prepared in swine (Accurate Chemical Co.) were added to the wells, and the plates were incubated and washed as before. Alkaline phosphatase substrate (1 mg of *p*-nitrophenylphosphate per ml in 0.01 M $MgCl_2$, 0.05 M Na_2CO_3, pH 9.8) was added to each well. After incubation at 22°C for 1 h, the reaction was stopped with 1 N NaOH. Portions of this reaction mixture were transferred to a microtiter plate, and the amount of colored *p*-nitrophenol was measured spectrophotometrically at 410 nm (A_{410}) with a Dynatech 600 plate reader (Dynatech Laboratories, Inc., Alexandria, Va.). The A_{410} is proportional to the total amount of cell-bound fimbriae per given cell mass (estimated by A_{650}). The A_{410}, corrected for viable cell concentration, is proportional to the quantity of fimbriae per CFU. The quantity of fimbriae on antibiotic-treated bacteria was expressed as a percentage of the fimbriae on untreated control bacteria.

Antibiotics. Trimethoprim was obtained from Hoffmann-La Roche, Inc. (Nutley, N.J.). The MIC was determined under conditions similar to those used for the antibiotic treatments (vide infra), except that cultures were incubated without shaking. Serial twofold dilutions of the antibiotic in TSB were inoculated with 2×10^7 log-phase bacteria and incubated statically at 37°C for 18 h. The lowest concentration of antibiotic that inhibited visible growth was defined as the MIC.

Antibiotic treatments. P fimbriate *E. coli* cells from an isolated colony on blood agar or tryptic soy agar were inoculated into TSB and incubated for 18 h in a 37°C shaking water bath. Fimbriation of this culture was checked by SA or latex carbohydrate agglutination. Fresh TSB was inoculated

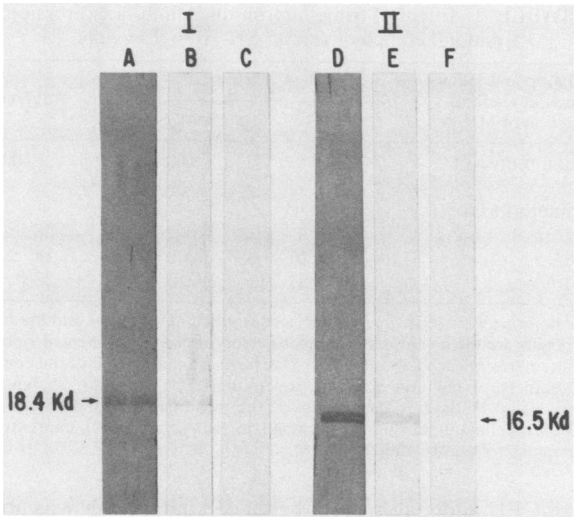

FIG. 1. Western immunoblot analyses of purified P (I) and type 1 (II) fimbriae probed with IgG-P (lanes B and C) or IgG-1 (lanes E and F). Lanes A and D were stained with Coomassie blue. Kd, Kilodaltons.

(1:20) and incubated for 1 h at 37°C with shaking. Trimethoprim solutions in TSB were added to samples of this culture to give final concentrations of 1/32, 1/16, and 1/8 MIC. The controls received TSB only. Incubation was continued for 3 h after the addition of antibiotic, unless otherwise specified and the A_{650} was measured. Bacterial cells were collected by centrifugation, washed twice with PBS, and resuspended with PBS to an A_{650} of 0.250 for the EIF and adherence assays. Viable bacterial counts were performed on adjusted cultures.

In vitro adhesion assays. *E. coli* adhesiveness was monitored by using the T24 human urinary bladder carcinoma cell line (2) (ATCC HTB-4) as described by Uhlin et al. (30). Nonconfluent monolayers of T24 cells (approximately 10^5 cells) on Thermolux (Miles Scientific, Dir. Miles Laboratories, Inc., Naperville, Ill.) cover slips in 24-well cluster dishes (Costar, Cambridge, Mass.) were obtained by culturing the cells for 20 h in modified McCoy medium (GIBCO) containing 10% fetal bovine serum (GIBCO) in an atmosphere of 5% CO_2.

For adhesion assays, spent medium was removed from the wells and replaced by bacterial suspensions containing 1×10^7 to 2×10^7 CFU/ml in fresh McCoy medium with HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (GIBCO). Bacteria were allowed to attach during 1 h of incubation at 37°C in a 5% CO_2 atmosphere. Plates were shaken two to three times during the incubation period. Each cover slip was washed extensively with modified McCoy medium (three washes of 1 ml each, 10 min per wash, on a rotary shaker), fixed with ice-cold methanol, and stained with Gram stain. The number of bacteria that adhered to 300 T24 cells was counted by light microscopy. The average number of bacteria per T24 cell relative to the number of CFU added was calculated. The adherence of bacteria grown in the presence of antibiotic was expressed as a percentage of the adherence of control bacteria grown without antibiotic.

RESULTS

Antifimbriae IgG. The specificity of anti-P-fimbrial IgG (IgG-P) and anti-type 1 fimbrial IgG (IgG-1) for P and type 1 fimbriae, respectively, was demonstrated by SA (Table 1), by Western blot (Fig. 1), and by EIF assay (Fig. 2). Strains HU849 and SH1-P (P fimbriae only) were agglutinated by IgG-P and not by IgG-1. Strain SH48 (type 1 fimbriae only) was agglutinated only by IgG-1. Strain SH-1 (both P and type 1 fimbriae) was agglutinated by both IgG-P and IgG-1. Nonfimbriate strain P678-54 and strain SH1-P grown at 18°C were not agglutinated by either IgG (Table 1). The EIF results agreed with the SA results; only P-fimbriate bacteria bound IgG-P, and only type 1-fimbriate cells bound IgG-1 (Fig. 2). Western blot analysis using purified fimbriae also demonstrated that IgG-P bound specifically to P fimbriae and IgG-1 bound specifically to type 1 fimbriae (Fig. 1).

Factors affecting expression of P and type 1 fimbriae. The effects of various growth conditions on the expression of P and type 1 fimbriae by *E. coli* SH1 and its variant, strain SH1-P, were monitored by SA, latex carbohydrate agglutination, and EIF assays as described in Materials and Methods. When strain SH1 bacteria were grown in TSB at 37°C with shaking, they expressed both P and type 1 fimbriae (Fig. 3). Strain SH1-P bacteria expressed P fimbriae, but not type 1 fimbriae, under these conditions. However, when cultures were incubated without shaking, SH1-P expressed both P and type 1 fimbriae. Neither P nor type 1 fimbriae were expressed by strain SH1 or SH1-P when cultures were incubated at 18°C. As has been shown for K99 (12), the quantity of P fimbriae per *E. coli* cell was greater after 4 h of incubation than after 18 or 24 h of incubation (results not shown). The effects of sublethal concentrations of antibiotics on P fimbria expression were determined by using 4-h cultures (1 h without and 3 h with antibiotic) of strain SH1-P grown to express only P fimbriae (vide supra).

Effects of antimicrobial treatments on bacterial growth and morphology. The MIC of trimethoprim for strain SH1-P was 4 μg/ml. The effects of 1/32, 1/16, and 1/8 MIC of trimethoprim on bacterial cell growth and morphology are shown in Table 2. The cell density (A_{650}) and viability (CFU per milliliter) of strain SH1-P were decreased in a dose-dependent manner after 3 h of incubation with trimethoprim. Bacterial cells were slightly elongated in the presence of 1/8

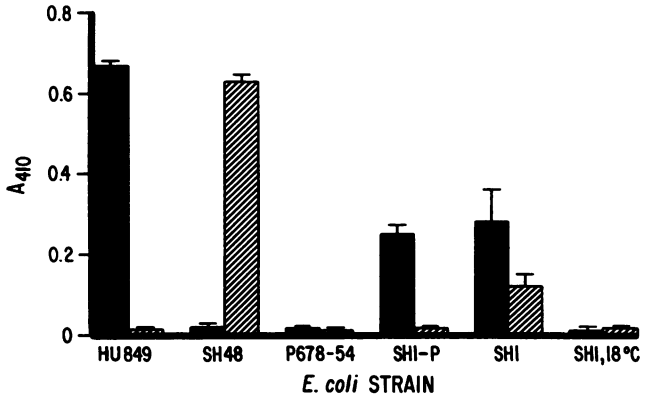

FIG. 2. Quantitation by EIF assay (see the text) of P (▬) and type 1 (▨) fimbriae on *E. coli* HU849 (P), SH48 (type 1), P678-54 (nonfimbriate), SH1-P (P), and SH1 (P and type 1) grown in TSB for 18 h at 37°C with shaking and on strain SH1 grown in TSB for 18 h at 18°C without shaking (18°C SH1; nonfimbriate). Results are expressed as the mean ± the standard error of the mean A_{410} for two experiments (HU849, SH48, and P678-54) or four experiments (SH1-P, SH1, and 18°C SH1). Measurements were in triplicate for each experiment. The A_{410} is proportional to the quantity of P fimbriae.

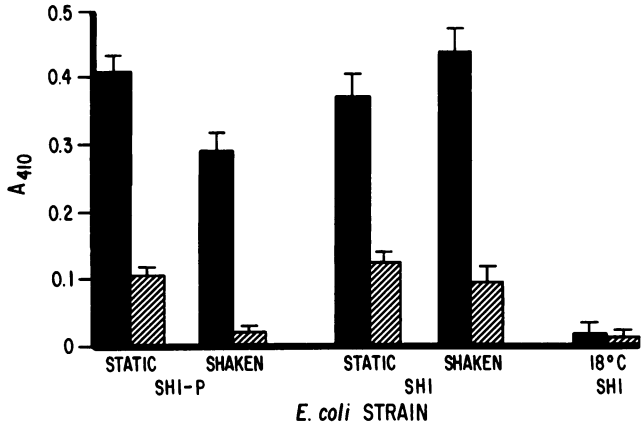

FIG. 3. Quantitation by EIF assay (see the text) of P (▬) and type 1 (▨) fimbriae on *E. coli* SH1-P and SH1 grown in TSB at 37°C for 18 h with and without shaking. The 18°C negative control was grown without shaking for 18 h. Results are shown as the mean ± the standard deviation of triplicate measurements.

MIC of trimethoprim, ranging from normal (i.e., equivalent to untreated bacteria) to five times normal length.

Effects of trimethoprim treatment on expression of P fimbriae. The effects of sub-MIC trimethoprim on the expression of P fimbriae by *E. coli* SH1-P are shown in Table 3. Total cell-bound P fimbriae on bacteria grown in 1/32, 1/16, or 1/8 MIC of trimethoprim was approximately 50% less than that of control bacteria grown without antibiotics. No dose response to the concentrations tested was seen. The amount of P fimbriae per CFU was also less for bacteria grown in 1/32 or 1/16 MIC of trimethoprim (48 and 70%, respectively). However, when bacteria were grown in 1/8 MIC of trimethoprim, the quantity of P fimbriae per CFU was 172% that of control bacteria. The quantity of P fimbriae on strain SH1-P bacteria grown at 18°C to suppress fimbriation was less than 6% of that of control bacteria.

Effects of trimethoprim treatment on adherence to uroepithelial cells. The effect of sub-MIC trimethoprim on the adherence of strain SH1-P bacteria to T24 bladder carcinoma cells is shown in Table 3. The average number of trimethoprim-treated bacteria per epithelial cell relative to the number of CFU added to the epithelial cell monolayer was about 20 to 30% of control adherence. As with P fimbria expression, no dose response was demonstrated. The adherence of nonfimbriate (18°C culture) strain SH1-P bacteria was less than 1% of control adherence.

TABLE 2. Effects of trimethoprim on cell growth (A_{650}), viability (CFU per milliliter), and morphology (length) of *E. coli* SH1-P

Antibiotic and initial concn in medium (fraction of MIC)[a]	% of control A_{650} after cultivation with antibiotic for[b]:		10^{-8} CFU/ml[c]	Bacterial length (μm)[d]
	3 h	24 h		
None (control)	100	100	3.8 ± 0.1	2–4
Trimethoprim				
1/32	76 ± 4	96 ± 0	3.4 ± 0.2	2–4
1/16	56 ± 1	85 ± 0	2.7 ± 0.1	2–4
1/8	38 ± 2	68 ± 9	1.0 ± 0.4	2–15

[a] Antibiotic was added to a 1-h log-phase culture ([4.4 ± 0.3] × 10^7 CFU/ml).
[b] A_{650} is expressed as a percentage of the control value ± 1 standard deviation. The data are from two experiments.
[c] The mean numbers of CFU per milliliter of a 4-h culture adjusted to an A_{650} of 0.250 ± 1 standard deviation (average of two experiments) are shown.
[d] Bacterial length was estimated by phase-contrast microscopy after 3 h of incubation with antibiotic.

TABLE 3. Effect of trimethoprim on P fimbria expression and adherence of *E. coli* SH-1P to T24 cells

Antibiotic and initial concn in medium (fraction of MIC)	% of P fimbriae[a]		Adherence[b]
	Total	Per CFU	
None (control)	100	100	100
Trimethoprim			
1/32	42 ± 1	48 ± 8	28 ± 16
1/16	51 ± 6	70 ± 8	18 ± 18
1/8	46 ± 1	172 ± 9	22 ± 5

[a] P fimbriae were measured by EIF as described in Materials and Methods. The results are the mean (± the standard error) of two experiments (triplicate measurements in each experiment), expressed as a percentage of the control.
[b] Numbers of bacteria adhering to cultured T24 cells are shown (see Materials and Methods). The results are the mean (± the standard error) of two experiments (duplicate measurements in each experiment), expressed as a percentage of control adherence.

DISCUSSION

The correlation of the adherence of *E. coli* to T24 bladder carcinoma cells and expression of P fimbriae has been shown previously (4, 30). The ability of strain SH1-P to adhere to T24 cells, the inability of nonfimbriate UPEC to adhere to these cells (less than 1% of P fimbriate bacterial adherence), and the ability of purified P fimbriae to inhibit the adherence of P fimbriate bacteria (results not shown) are consistent with P fimbrial mediation of the adherence of *E. coli* SH1-P to uroepithelial cells. We have described herein an EIF assay to measure the expression of P fimbriae and application of the assay to examination of the effects of trimethoprim on expression of P fimbriae. Effects on P fimbriation correlate with the effects on adhesion to T24 epithelial cells. Understanding the mechanism of this interference will provide information on bacterium-host interactions important in the pathogenesis of urinary tract infections. In addition, this assay might be useful for identifying antibiotics that may have antiadhesive activity. By interfering with the expression of P fimbriae by UPEC, sublethal doses of antimicrobial agents might interfere with the adherence of these bacteria to the uroepithelial mucosa and in this way prevent colonization and infection.

Several studies have reported decreased bacterial adherence to epithelial cells (24, 25, 31, 33), decreased hemagglutination (14, 32, 34), or decreased fimbriation (21, 28, 32) after treatment of UPEC with sub-MICs of trimethoprim. For this reason, trimethoprim was selected to validate the utility of the assay in measuring changes in fimbriation and determine the correlation of the effect on P fimbriation with the effect on bacterial adherence. Trimethoprim was not tested above a concentration of 500 μg/ml (1/8 MIC), since effects on bacterial growth, P fimbriation, and bacterial adherence to T24 cells were observed at lower concentrations (1/32 to 1/8 MIC), and higher concentrations caused filament formation. Difficulties encountered in interpreting results obtained with filaments after antibiotic treatments have been described previously (22) and were also encountered after cephalosporin treatment (unpublished results).

In the original enzyme-linked antibody centrifuge assay described by Isaacson (12), K99 fimbria expression was

quantitated as fimbriae per viable cell. In the EIF assay described in this study, total P fimbriae per standardized suspension (A_{650}, 0.250) of washed bacteria was determined to be a better method for quantitation of antibiotic-treated bacteria because it was less affected by morphologic changes (23). Total P fimbriae correlated better with bacterial adherence to T24 cells than did P fimbriae per CFU in the presence of filament formation (Table 3). Elongated bacteria may have more fimbriae per CFU, even when total fimbria expression is reduced. For example, after treatment with 1/8 MIC of trimethoprim, SH1-P bacteria were two to five times longer than untreated bacteria. If the treated bacteria had the same amount of P fimbriae per given surface area as did control bacteria, the amount of P fimbriae per CFU should also be two to five times that of the control, rather than increased by 70% as observed by us. The adherence results, however, are consistent with the 50% decrease seen in total P fimbriation. Although these results are also consistent with a lower density of fimbriae on the cell surface, the question of whether filaments require more or less fimbriae per surface area to bind to epithelium has not been addressed.

With the EIF assay, it was possible to independently quantitate effects of trimethoprim on the expression of either P or type 1 fimbriae by UPEC strain SH1 bacteria that produced both fimbrial types. Expression of more than one fimbrial type by UPEC is a common phenomenon (15, 20), and the effects of growth conditions on expression of P and type 1 fimbriae have been previously described (8). The effects of trimethoprim on the expression of P fimbriae by strain SH1 (results not shown) were similar to the effects on strain SH1-P (Table 3). Strain SH1 could not be used in the adherence assays because the type 1 fimbriae also affected adherence to T24 cells. Trimethoprim treatment did not decrease type 1 fimbriation (results not shown).

In addition to affecting fimbria expression, antimicrobial treatments might also affect the release of fimbriae from the bacterial surface. Fimbriae released by antimicrobial treatments could further interfere with bacterial adherence to epithelium by competing with fimbriate bacteria for binding sites. Only cell-bound fimbriae were measurable by the EIF assay.

The EIF assay is a simpler, faster, and more efficient technique than the enzyme-linked antibody centrifuge assay. The total assay time is shortened because immobilization of the bacteria and the wash steps are simpler and faster by filtration than by centrifugation. The 96-well microtiter design makes the EIF assay suitable for testing large numbers of samples at one time.

The EIF assay can be used to quantitate the effects of sublethal doses of antimicrobial agents on the expression of fimbriae by *E. coli*. The ability to quantitate the effects on P fimbriation in the presence of other fimbriae (e.g., type 1 fimbriae) is an advantage of this assay in studies on the role of this fimbria in the pathogenesis of urinary tract infections. This assay can be used to identify antimicrobial agents with the potential to function as antiadhesins.

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