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Increased Expression of Type-1 Fimbriae by Nonpathogenic *Escherichia coli* 83972 Results in an Increased Capacity for Catheter Adherence and Bacterial Interference

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Abstract

Background—In vitro, urinary catheter colonization by avirulent *Escherichia coli* 83972 impedes subsequent catheter colonization by a variety of uropathogenic organisms. However, *E. coli* 83972 shows a low efficacy of adherence to silicone urinary catheter material, possibly because the *fim* operon encoding adhesive type 1 fimbriae is incomplete. We hypothesized that improving the catheter adherence of *E. coli* 83972 would improve its bacterial interference properties.

Methods—We created adhesive mutants by transforming wild-type *E. coli* 83972 with *fim*⁺ plasmids. Adherence to urinary catheters and ability to prevent uropathogenic *E. coli* from colonizing urinary catheters were studied by use of a sonication assay.

Results—The addition of a single-copy *fim*⁺ plasmid increased adherence to urinary catheters 10-fold, and addition of an 18-copy *fim*⁺ plasmid increased adherence 100-fold. The more adherent 18-copy *fim*⁺ plasmid strain was more effective at blocking catheter colonization by pathogenic *E. coli* than was the wild-type parental strain. Neither Δ *fim* nor *fim*⁺ *E. coli* 83972 adhered to shed urinary epithelial cells.

Conclusions—Our results indicate that improving urinary catheter adherence augments the bacterial interference capabilities of benign *E. coli* 83972. Increased expression of type-1 fimbriae may enhance bacterial interference without conferring virulence on *E. coli* 83972.

Each year, more than 30 million urinary catheters are used for patients in the United States [1]. Urinary tract infection (UTI) accounts for over 30% of all nosocomial infection in US intensive care units, and 97% of these UTIs occur in catheterized patients [2]. More than 1 million catheter-associated UTIs (CA-UTI) are reported annually by infection control surveillance [3]. Persons with a catheterized urinary tract acquire bacteriuria at the rate of about

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3%–10% per day, and prolonged catheterization is inevitably accompanied by bacteriuria [4]. Complications of chronic bacteriuria include prostatitis, epididymitis, scrotal abscesses, catheter blockage from encrustation, pyelonephritis, and bacteremia [5].

The catheter-associated biofilm of adherent organisms and secreted polysaccharide matrix is central to the pathogenesis of CA-UTI [6]. Compared to their planktonic (i.e., free-floating) counterparts, organisms in bio-films are less susceptible to antimicrobial agents and host defenses [7]. The use of standard antimicrobial agents to sterilize the urine of individuals undergoing prolonged catheterization leads to bladder colonization with antimicrobial-resistant organisms [8–10]. No strategy effectively prevents biofilm formation on long-term, indwelling urinary catheters [11].

Bacterial interference, or the use of benign bacteria to prevent infection by virulent pathogens [12,13], may offer a solution to the problem of CA-UTI. Three small trials involving persons with spinal cord injury and deliberate bladder colonization with nonpathogenic *Escherichia coli* (*E. coli* 83972) have demonstrated an association between colonization with this nonpathogenic organism and decreased incidence of UTI [14–16]. In vitro, precoating urinary catheters with a biofilm of *E. coli* 83972 resulted in a decrease in catheter colonization by uropathogens [17,18]. These promising results support our interest in exploring the mechanism of bacterial interference employed by *E. coli* 83972 so that we can optimize and exploit this interaction.

The adherence of bacterial cells to a surface is an essential early step in biofilm formation [19]. Type-1 fimbriae, encoded by the *fim* operon, are considered a major mediating factor in *E. coli* surface adherence [20]. Wild-type *E. coli* 83972 was recently discovered to have a major deletion of 4.5 kb between *fimB* and *fimD* that removes the gene encoding the major structural component of type-1 fimbriae, *fimA* [21]. Although the *fimH* gene encoding the adhesive component of type-1 fimbriae is intact, it is very unlikely that wild-type *E. coli* 83972 expresses functional type-1 fimbriae. We hypothesized that introducing a complete *fim* operon into *E. coli* 83972 might increase its ability to adhere to urinary catheters, which might, in turn, improve its capacity for bacterial interference.

MATERIALS AND METHODS

Adherence of *E. coli* isolates to silicone

Silicone is one of the predominant surface materials for the urinary catheters used in clinical settings. The capacity of clinical and laboratory isolates of *E. coli* to adhere to squares of siliconized latex (DaPro) after 48 h of shaking at 37°C in synthetic urine [22] was measured quantitatively by using a wash and sonication assay. Clinical uropathogenic isolates were obtained from the authors' strain collection (R.A.H. and S.I.H.). GR12 and J96 are well characterized uropathogenic strains [23].

Bacterial strains and plasmids

The *E. coli* 83972– derivative strains and plasmids used in this study are listed in table 1. The construction of pSH2 has been described elsewhere [24,25]. In brief, pSH2 carries the chromosomal *fim* operon (11.2 kb) of *E. coli* J96 in the *SalI* site of the tetracycline-resistance gene on pACYC184 [26]. *E. coli* J96 was chosen as the source for the *fim* operon because it adhered to silicone catheter material better than 8 other *E. coli* isolates (figure 1), and because the *fim* operon is known to be functional in this organism [27]. Plasmid RHU2602 was created by cutting pACYC184 with *HincII* and religating, thus removing a major portion of the tetracycline-resistance gene. Plasmid RHU2642 was created by ligating the purified fragment of pFOS1 [26] cut with *HindIII* and *HpaI* with the purified fragment of pSH2 cut with *HindIII*

and *PshAI*. Plasmid RHU2607 contains the *Sall* fragment from pSH2 inserted into the *Sall* site of pBCKS [29,30].

Because the chromosomally located *fimH* in wild-type *E. coli* 83972 appears to express a functional product [21], we transformed *E. coli* HU2222 with pRHU2602 to create HU2634. HU2222 is *E. coli* 83972 with a 102-bp deletion in *fimH*, including the start codon, and an 800-bp deletion in *papG* (in the P pilus operon, relevant for pyelonephritis) [31]. All other derivatives used in this study (HU2603, HU2651, HU2545, and HU2610) were created by transforming wild-type *E. coli* 83972. All derivative strains of 83972 were resistant to chloramphenicol and were sensitive to tetracycline. The uropathogenic challenge strain, *E. coli* pathogenic (ECP), was a lactose-negative *E. coli* isolate from a patient with symptomatic CA-UTI. This clinical isolate was modified by transformation with pACYC184, thus conferring resistance to both chloramphenicol and tetracycline

Plasmid quantitation

Plasmid DNA was extracted from an equivalent number of bacterial cells from each strain (QIA-prep Miniprep; Qiagen). Plasmids were cut once to linearize with *HindIII* and then electrophoresed through a 0.7% gel. AlphaDigiDoc RT 2 software (Alpha Innotec) was used to quantitate the DNA in each gel band.

Hemagglutination assay

We measured hemagglutination titers of guinea pig erythrocytes as a functional assay for expression of type-1 fimbriae [32–34]. Each strain was grown overnight in 50 mL of Luria-Bertani (LB) broth plus chloramphenicol 20 µg/mL, rocking, at 37°C. Cells were harvested by centrifugation and resuspended in PBS. Each strain was adjusted to an OD₆₀₀ of 1.0. Serial 2-fold dilutions of 50 µL of bacteria were suspended in round-bottom polystyrene plates (Corning). Each well also contained 25 µL PBS and 50 µL of 5% guinea pig erythrocytes (Cambrex). Plates were incubated, rocking, at 4°C for 2 h. The hemagglutination titer was determined as the lowest dilution that prevented formation of a defined pellet of erythrocytes. In some experiments, 50 mmol/L mannose was added.

Quantitative real-time polymerase chain reaction (PCR)

Quantitative real-time PCR was used to confirm differential expression of *fimH* by the mutant strains of *E. coli* 83972. Strains were grown to an A₆₀₀ of 1.0. RNA was isolated using the protocol of the RNeasy Mini Kit (Qiagen) and was treated with DNase to remove any contaminating DNA. The quantity and quality of RNA was determined using an Agilent 2100 Bioanalyzer. The cDNA were synthesized by use of the SuperScript III reverse transcriptase (RT) protocol (Invitrogen) with the random primers (catalog no. 48190 – 011; Invitrogen). The cDNA was then diluted with nuclease-free water to obtain a final dilution of 1:10 for use in the real-time PCR. Diluted cDNA template (5 µL) was added to a 96-well PCR plate (Applied Biosystems). Taqman gene expression master mixtures were prepared for each primer set in accordance with the manufacturer's protocol (Applied Biosystems). A 2-step program was used with a 7300 real-time PCR system (Applied Biosystems). The 2^{-ΔΔCT} method was used for analysis of *fimH* mRNA expression by the various *E. coli* 83972- derivative strains in comparison with expression by the Δ*fimH* strain (HU2634 as the calibrator), with *16S* as the normalizing gene [35]. The primer sequences were as follows: fimH-F, 5'-TGGCGATTAAAGCTGGCTCATTA-3'; fimH-R, 5'-CACAACTGGAAATCATCGCTGTTA-3'; fimH-M2 (FAM), 5'-TCTGTCGCAAAATAAGF-3'.

Catheter adherence assay

The ability of the 5 derivative strains of *E. coli* 83972 to adhere to urinary catheters was assessed with the following assay. All-silicone urinary catheters (Silicone Foley Catheters; Rochester Medical) were cut into 7-cm pieces. Each catheter piece was added to a 50-mL centrifuge tube that contained 20 µg/mL chloramphenicol in LB broth (Difco Laboratories) and inoculated with 10⁵ cfu/mL of a given strain (50 µL of a suspension with an A₆₀₀ of 0.2–0.3 were added to 50 mL of LB in each tube). Catheter pieces were incubated with *E. coli*, rocking, for 24 h at 37°C. Segments were rinsed and flushed with PBS. Three 1-cm segments were cut from each catheter, and each was sonicated for 10 min in 1 mL PBS plus 0.01% sodium-dodecyl sulfate SDS [36]. Dilutions of the sonicates were plated, incubated overnight, and counted to determine the number of colony-forming units per 1-cm catheter segment. The median of the colony-forming unit counts for the 3 segments was used as the value for each catheter. Experiments were repeated 7 times and results were analyzed by using the Wilcoxon rank sum test. The role of type-1 fimbriae in catheter adherence was confirmed by repeating the adherence test with strain HU2545 in the presence and absence of 50 mmol/L mannose.

Bacterial interference assay

Interference trials were performed by using our established protocol for in vitro interference [17,18]. In brief, catheters were allowed to incubate overnight with *E. coli* 83972 or a derivative strain in LB broth to establish a biofilm. They were then exposed to the uropathogenic challenge strain ECP in LB broth for 30 min and then were transferred to sterile urine for a further 48 h of incubation with a change of media at 24 h. Bacteria adhering to the catheters were harvested by rinsing followed by sonication. Biofilm growth occurred in the presence of chloramphenicol 20 µg/mL throughout the experiments [37]. *E. coli* 83972 strains (*lac*⁺) and ECP (*lac*[−]) were distinguished by their appearance on MacConkey agar and/or by their ability to grow on plates containing tetracycline. Each experiment was repeated 4–7 times, and the numbers of pathogenic *E. coli* recovered from the catheters were compared using the Wilcoxon rank sum test.

Biofilm imaging

E. coli in LB broth plus chloramphenicol was incubated, rocking, at 37°C for 48 h in a chambered cover-glass (Lab-Tek; Nalge Nunc International). This device consisted of 2 plastic media chambers mounted on a #1 borosilicate coverglass with a cover. Each strain was inoculated in 2 chambers, and the media was changed at 24 h. After 48 h of incubation, chambers were rinsed once with PBS and then stained with 50 µL of 20 mmol/L DRAQ5 (Biostatus Limited). Images were obtained with a Zeiss LSM 510 scanning confocal microscope using the 100× oil objective and the HeNe2 laser (633 nm). Images were processed with Zeiss LSM 5 software. Five randomly chosen fields were imaged for each strain. Bacterial cell counts were obtained with MetaMorph software (version 6.3r7; Molecular Devices) and compared by using the Kruskal-Wallis version of analysis of variance (ANOVA) to compare medians across groups (SigmaStat 3.5; Systat Software).

Uroepithelial cell adherence

For these experiments, we used bacterial strains HU2603 (wild type), HU2545 (most adherent), and *E. coli* CFT073 (positive control), and we modified the method of Svanborg Eden et al. [38]. The sediment from first morning urine from a female donor was washed in buffered saline gelatin (BSG) and resuspended. The number of cells per milliliter was calculated by direct-light microscopy. Pellets containing 2 × 10⁵ uroepithelial cells/mL were resuspended in 1 mL BSG with 5 × 10⁹ *E. coli*. Bacteria and cells were incubated together for 2 h at 37°C, rocking, then washed 4 times in BSG and stained with 20 µmol/L DRAQ5 (Biostatus Limited), a far-red, fluorescent DNA dye. Cells were imaged by using light and fluorescence microscopy, and

the number of bacteria adhering to 80 cells was counted for each strain, up to a maximum of 100 adherent bacteria/cell.

RESULTS

Adherence of *E. coli* isolates to silicone

E. coli 83972 had a low efficacy of adherence to silicone urinary catheter material, compared with other *E. coli* strains, including uropathogenic clinical isolates and laboratory strains (figure 1). The adherence capacity of *E. coli* 83972 was approximately 10-fold less than that of J96.

Plasmid DNA quantitation, hemagglutination, and *fimH* expression

The amount of plasmid DNA per cell increased as plasmid copy number increased, as expected (figure 2A). Hemagglutination, a marker for type-1 fimbriae expression, was increased in a nonlinear fashion by adding plasmid-encoded *fim* (figure 2B). HU2545 and HU2610 both hemagglutinated guinea pig erythrocytes at a 1:16 dilution, while HU2651 did not hemagglutinate beyond a 1:2 dilution. HU2634 and HU2603 did not hemagglutinate in this assay. Adding 50 mmol/L mannose to the media reduced the hemagglutination titer of the 18-copy HU2545 by 8-fold, from 1:32 to 1:2. These results demonstrate that the plasmid-encoded *fim* was expressed in 83972, and the increased plasmid copy number correlated with increased expression of type-1 fimbriae. Quantitative real-time PCR data showed that *fimH* gene expression was correlated with the predicted copy number of the *fimH* gene (figure 2C).

Catheter adherence

Adherence to silicone urinary catheters was increased by adding a *fim*-containing plasmid (figure 3). The median baseline rate of catheter adherence for the wild-type strain (HU2603) and the *fimH* deletion strain (HU2634) was 10^4 cfu per 1-cm catheter segment. The median rate of adherence was increased 10-fold by adding the single-copy *fim* plasmid (strain HU2651) and 100-fold by adding the 18-copy *fim* plasmid (strain HU2545). In the absence of mannose, 8×10^5 cfu of HU2545 per 1-cm catheter segment were recovered, whereas in the presence of 50 mmol/L mannose, we only recovered 3×10^3 cfu of HU2545 per 1-cm segment.

The median adherence rate of the 75-copy *fim* plasmid derivative (strain HU2610) was less than that of the 18-copy derivative (strain HU2545). We also noted that after 5 h of incubation, HU2610 was >0.5 log less abundant than the other 4 strains (5.5×10^6 vs. 1.6×10^7 – 2.3×10^7 cfu/mL, data not shown). To investigate whether the decreased growth rate of HU2610 could account for its decreased rate of catheter adherence in comparison with HU2545, the data from figure 3 were normalized by dividing the number of adherent organisms recovered from each catheter by the number of planktonic organisms in the media. The relationship between the relative adherence rates of HU2545 and HU2610 did not change (data not shown).

Biofilm imaging

Confocal microscopy imaging of the biofilms formed by these 5 derivative strains was in agreement with the results of the catheter adherence experiments (figure 4). The median number of cells per high-power field was 28 for HU2634, 55 for HU2603, 417 for HU2651, 656 for HU2545, and 505 for HU2610.

Bacterial interference

The pair of *E. coli* 83972 strains chosen for comparison was HU2603 (wild type) and HU2545 (the most adherent strain). The difference in the recovery of ECP in the presence and absence of a preexisting biofilm is a measure of bacterial interference. In all experiments, the presence of a pre-existing biofilm of a strain of *E. coli* 83972 (either HU2603 or HU2545) significantly

decreased the median numbers of ECP recovered from the urinary catheter (figure 5). After 48 h of incubation, fewer pathogens were recovered from catheters on which the preexisting biofilm was composed of the more adhesive strain (HU2545), compared to catheters with a biofilm composed of the less adhesive strain (HU2603) ($P = .021$, by the Wilcoxon rank sum test).

Uroepithelial cell adherence

Adherence of HU2603 (wild-type *fim* strain) and HU2545 (the most adhesive *fim*⁺ strain) to bladder uroepithelial cells was low, in contrast to *E. coli* CFT073 (figure 6). For *E. coli* CFT073, the average number of adherent bacteria per cell was 59 (median, 65), in contrast to the average of 2 for HU2603 (median, 0) and 1.6 for HU2545 (median, 0) ($P < .001$, Kruskal-Wallis ANOVA).

DISCUSSION

The importance of type-1 fimbriae for the adherence of *E. coli* to abiotic surfaces such as polyvinyl chloride plates has been well documented [20,39], but this study confirms a role for type-1 fimbriae in adherence to a clinically relevant material: silicone urinary catheters. Our results indicate that defective expression of type-1 fimbriae plays a role in the decreased rate of catheter adherence observed for *E. coli* 83972. This defect can be corrected by adding intact *fim* operon on a plasmid. The hemagglutination assays and mannose inhibition assays support the hypothesis that the observed increase in adherence rate arises from increased surface expression of type-1 fimbriae. More copies of the *fim* operon result in better adherence, up to a point. Type-1 fimbriae overexpression by the 75-copy plasmid in HU2610 did not result in an improved rate of adherence, in comparison to the rate for the 18-copy plasmid in HU2545. The decreased growth rate of HU2610 did not appear to account for its decreased adherence rate in comparison to that of HU2545, because the relationship of their relative adherence rates did not change when normalized by planktonic growth. Perhaps in strain HU2610 the overexpression of *fim* does not lead to increased surface expression of type-1 fimbriae. The similar hemagglutination titers of strains HU2545 and HU2610 support that explanation.

We did not observe any differences in adherence rates between strains HU2634 and HU2603. These strains are isogenic except for the deletions in *fimH* and *papG* in HU2634. Our results suggest that the presence of an intact *fimH* in wild-type 83972 is not sufficient for expression of a functional gene product in the absence of the remainder of the *fim* operon.

Improved rates of catheter adherence resulted in better bacterial interference with respect to urinary catheter colonization by a pathogen. Better adherence rates may allow the preexisting biofilm to block uropathogens' access to the catheter surface through spatial constraints. The preexisting biofilm could also impair catheter colonization by pathogens through production of extracellular matrix, consumption of nutrients, changes in the local microenvironment, and production of quorum inhibitors. A greater number of organisms in the preexisting biofilm would be expected to enhance any of these interference mechanisms. Gene expression studies in dual species biofilms (*E. coli* 83972 and uropathogens) may elucidate which of these factors are important to the observed interference effect.

In the clinical setting, improved rates of catheter adherence may translate to better bacterial persistence in the bladder. Enhanced interference and persistence are desirable traits for the organism that we use in our clinical trials of bacterial interference for the prevention of CA-UTI. Ideally, we will be able to modify the adhesive component of the type-1 fimbriae, FimH, so that it adheres better to catheters without adhering well to urinary epithelial cells and thus becoming virulent. Previous work suggests that such a modification is possible, as FimH mutants have been described that have enhanced capacities to form biofilms yet are less

susceptible to mannose inhibition than wild-type FimH [20]. Because the normal bladder epithelial cell ligand for FimH is mannose [40], such mutants might have lost their ability to adhere to bladder epithelial cells. Furthermore, we found that strain HU2545, despite its mannose-sensitive adherence to urinary catheter material, did not adhere significantly to bladder epithelial cells. This finding is in accord with those of other researchers, indicating that type-1 fimbriae expressed in the genetic background of *E. coli* 83972 do not promote mucosal inflammation in the human urinary tract [41]. These results have important implications for our clinical work, as they suggest that our goal of improving the capacity of *E. coli* 83972 for catheter adherence and bacterial interference can be achieved without increasing the organism's virulence.

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Figure 1. Adherence of *Escherichia coli* 83927 to silicone, compared with the adherence of other *E. coli* isolates. The results are the mean of duplicate assays.

Figure 2.

Plasmid copy number and *fim* expression. **A**, Plasmid quantitation. Plasmid DNA was extracted from an equivalent number of *Escherichia coli* cells from each strain. **B**, *fim*⁺ plasmids conferred increased hemagglutination. *Black diamonds*, hemagglutination titers; *white squares*, ng DNA. When reciprocal hemagglutination titers were plotted against plasmid copy number, a nonlinear relationship was seen. **C**, Quantitative real-time polymerase chain reaction of *fimH* gene expression in the 5 strains. Gene expression of *fimH* was measured by the $2^{-\Delta\Delta C_T}$ method [35], in which *16S* was used as the normalization gene and the *fimH* deletion mutant (HU2634) was used as the calibrator. The data represent the mean of 3 duplicate experiments.

Figure 3.

Plasmid-encoded *fim* and adherence to urinary catheters. The addition of plasmid-encoded *fim* (3 strains on right) increased the strains' median rate of adherence to catheters, in comparison with that of *fimH* deletion and wild-type strains (2 strains on left). $P < .05$ for the following comparisons: HU2634 to HU2651, HU2545, and HU2610; HU2603 to HU2651, HU2545, and HU2610; HU2651 to HU2545; and HU2545 to HU2610 (Wilcoxon rank sum test). The following comparisons were not statistically significant: HU2634 to HU2603 and HU2651 to HU2610 ($P > .05$, by the Wilcoxon rank sum test).

Figure 4.

Confocal microscopy analysis of biofilm formation. *A*, Representative images of 24-h biofilms formed by the 5 derivative strains. *B*, The adherent cell counts of the 5 strains varied significantly at 48 h ($P < .001$, Kruskal-Wallis analysis of variance). Results are the medians of 5 microscopic fields for each strain. The main source of variance was the fact that HU2545 had higher cell counts than HU2634 or HU2603 ($P < .05$, Tukey test).

Figure 5.

Addition of the 18-copy *fim* plasmid and interference with pathogenic *Escherichia coli* (ECP). Data are the median number of ECP recovered from the catheter surface in 5 experiments. The presence of a preexisting biofilm of *E. coli* HU2603 or HU2545 significantly decreased the number of ECP recovered from the catheter surface ($P \leq .05$ for the comparison of ECP alone and the combination of HU2603 and ECP, and for the comparison of ECP alone and the combination of HU2545 and ECP). Also, smaller numbers of ECP colonized catheters that had been precoated with the more adherent strain HU2545, compared with catheters precoated with the wild-type strain HU2603 ($P = .021$ for comparison of the combination of HU2603 and ECP with the combination of HU2545 and ECP).

Figure 6.

Adhesion of derivative strains of *Escherichia coli* 83972 to bladder uroepithelial cells. Images of uroepithelial cells and bacteria stained with DRAQ5 were acquired by using the 63× objective of a Zeiss Axioplan II upright microscope with a CoolSnap HQ CCD camera. Light images were obtained by use of differential interference contrast (DIC) prisms (left column), and fluorescent images were obtained by using Texas Red wavelength and filters (center column). DIC and fluorescent images were merged (right column) by using MetaMorph software.

Table 1Derivative strains of *Escherichia coli* 83972.

Derivative strain	Chromosomal <i>fim</i> genotype	Plasmid	Predicted plasmid copy number of <i>fim</i>
HU2634	$\Delta fimH$	pRHU2602 (pACYC184 Δ Tc)	0
HU2603	Wild-type	pRHU2602 (pACYC184 Δ Tc)	0
HU2651	Wild-type	pRHU2642 (pFOS1, <i>fim</i> ⁺)	1 [26]
HU2545	Wild-type	pSH2 [27] (pACYC184, <i>fim</i> ⁺)	18 [28]
HU2610	Wild-type	pRHU2607 (pBCKS, <i>fim</i> ⁺)	75 [29,30]