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***Escherichia coli* 83972 INHIBITS CATHETER ADHERENCE BY A BROAD SPECTRUM OF UROPATHOGENS**

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Abstract

Objectives—The presence of a nonpathogenic organism on the surface of a urinary catheter might impede catheter colonization by pathogens and thus prevent urinary tract infection. Previously, we reported that preinoculating urinary catheters with a nonpathogenic strain of *Escherichia coli* (83972) significantly impeded catheter colonization in vitro by gram-positive bacteria (*Enterococcus faecalis*). To explore this phenomenon further, we investigated in vitro whether *E. coli* 83972 could likewise inhibit catheter colonization by gram-negative and fungal uropathogens (*Providencia stuartii*, uropathogenic *E. coli*, and *Candida albicans*).

Methods—For each of the three uropathogens tested, we examined three different incubation conditions: (a) *E. coli* plus uropathogen catheters were exposed to *E. coli* 83972 for 24 hours and then to a uropathogen for 30 minutes; (b) *E. coli*-alone catheters were incubated with *E. coli* for 24 hours and then in sterile broth for 30 minutes; and (c) uropathogen-alone catheters were incubated in sterile broth for 24 hours before the 30-minute incubation with the uropathogen. All catheters were subsequently incubated in sterile human urine for 24 hours. Catheters were then rinsed and sonicated to determine the numbers of adherent organisms per centimeter.

Results—Pre-exposure of the catheter to *E. coli* 83972 in all cases significantly reduced the number of uropathogens colonizing the catheter surfaces.

Conclusions—*E. coli* 83972 significantly impedes catheter colonization by all tested bacterial and fungal pathogens. The broad applicability of this particular approach to bacterial interference in vitro invites further exploration in vivo.

Most individuals with spinal cord injury (SCI) have a neurogenic bladder, and the resulting urinary stasis and bladder catheterization predispose them to recurrent urinary tract infection (UTI). Now that individuals with SCI have normal or near-normal life expectancies, secondary infectious complications, such as UTI, have become major causes of morbidity and mortality.¹ Currently, few, if any, measures are effective at preventing UTI in persons with a neurogenic bladder and an indwelling urinary catheter.

Bacterial interference, or using nonpathogenic bacteria to prevent symptomatic infection,² may offer a solution to the problem of recurrent UTI in chronically catheterized individuals. Essentially, all patients with an indwelling urinary catheter for 30 days or longer have bacteriuria, frequently with more than one organism.^{3–5} Studies in elderly subjects, patients with SCI, and adult women indicate that treatment of asymptomatic bacteriuria in these populations is neither beneficial nor effective.^{2,6–8} Allowing asymptomatic bladder

colonization to persist in order to prevent colonization by a pathogenic strain may constitute a passive approach to bacterial interference.²

A more active approach to bacterial interference involves intentionally colonizing the bladder with a nonpathogenic organism to prevent infection with more virulent bacteria. Prospective clinical trials have demonstrated that direct bladder inoculation with a nonpathogenic strain of *E. coli* (83972) is safe and protective in persons with SCI.^{9,10} However, the direct bladder inoculation process was cumbersome and frequently required multiple attempts to induce colonization. Furthermore, we wished to address the problem of recurrent UTI at the level at which catheter-associated UTI begins—the formation of biofilm. Implanted urinary catheters rapidly acquire a complex, three-dimensional biofilm composed of bacteria, their extracellular products, and components deposited from bodily fluids. The pathogenic organisms in a biofilm continually seed the bladder, leading to bacteriuria and/or UTI.³ Because biofilm formation on a wet-implanted device such as a urinary catheter is nearly impossible to prevent,^{11,12} we propose instead to manipulate the adherent microbial flora. We ultimately plan to use preinoculated Foley catheters as a means to colonize the human bladder with *E. coli* 83972 and thus prevent UTI.

With this goal in mind, we investigated the interference capacities of *E. coli* 83972 in vitro. As we have previously reported, preinoculating urinary catheters with *E. coli* 83972 significantly impeded catheter colonization by the gram-positive organism *Enterococcus faecalis*.¹³ To explore this interference phenomenon further, we investigated in vitro whether *E. coli* 83972 could likewise inhibit catheter colonization by gram-negative and fungal uropathogens, including *Providencia stuartii*, uropathogenic lactose-negative *E. coli*, and *Candida albicans*. These three organisms were specifically chosen because they are major causes of bacteriuria and/or UTI in chronically catheterized persons.^{14,15}

MATERIAL AND METHODS

Bacteria

E. coli 83972, serotype ON:KN (N = nontypeable), lactose positive, was initially isolated from the urine of a young girl who had had asymptomatic bacteriuria for 3 years with stable renal function.¹⁶ This bacterial strain was selected because of its documented ability to persist in the urinary tract without causing disease. The *P. stuartii*, uropathogenic lactose-negative *E. coli*, and *C. albicans* strains were isolated from the urine of patients with symptomatic UTI. All strains were stored at -80°C in a 10% glycerol culture. Bacterial strains were then passaged on either trypticase soy agar with 5% sheep blood (BBL, Cockeysville, Md) or MacConkey agar (BBL). The *C. albicans* strain was passaged on Sabouraud agar (Becton Dickinson, Sparks, Md). Bacteria were retrieved from the agar plates and cultured overnight in trypticase soy broth (TSB; Becton Dickinson). A sample of the overnight culture was adjusted to $\sim 10^8$ colony-forming unit (cfu)/mL.

Catheter Preparation

Commercially available, 12F Foley catheters composed of latex with a hydrophilic coating were used in this study (Bardex, Covington, Ga). Under sterile conditions, the catheter shaft was cut into 7-cm pieces. The balloon lumen of each piece was plugged on both ends with sterile pipette tips and sealed with flame. The purpose of sealing the balloon channel was to leave only the main catheter lumen accessible to organisms, such as is the case when a catheter is placed in the human bladder. The catheter pieces were placed in sterile 50-mL centrifuge tubes containing TSB.

Experimental Variables and Controls

Each set of experiments included one experimental catheter (incubated first with *E. coli* 83972 and subsequently with a uropathogen), and two control catheters (one incubated only with *E. coli* 83972 and one incubated only with the uropathogen). Six to nine sets of experiments were performed with each of the three uropathogens tested. The three catheters in each set were incubated at 37°C as follows: (a) *E. coli* plus uropathogen catheters were exposed to *E. coli* 83972 in TSB for 24 hours at a starting concentration of 10^5 cfu/mL and then were transferred without washing to a tube containing a uropathogen in TSB at a concentration of 10^5 cfu/mL for 30 minutes; (b) *E. coli*-alone catheters were incubated for 24 hours with *E. coli* 83972 at a starting concentration of 10^5 cfu/mL and then transferred to sterile broth for 30 minutes; and (c) uropathogen-alone catheters were incubated in sterile broth for 24 hours before the 30-minute incubation with a uropathogen. After the 30-minute incubation step, all catheter pieces were removed, placed immediately in filter-sterilized human urine, and incubated in urine at 37°C for 24 hours. Thus, biofilm formation by the challenge uropathogens occurred in urine, as would occur in vivo. The urine was obtained from a single male donor and was filter sterilized by passage through a 0.22- μ m filter.

The purpose of incubating the *E. coli* 83972-coated catheters for 30 minutes with uropathogens in broth was to standardize the catheter exposure to the uropathogens. Because the organisms used in these trials grow better in broth than in urine, catheter seeding by both *E. coli* 83972 and the challenge uropathogens was performed in broth (TSB).

Catheter Harvest

Under sterile conditions, the 7-cm catheter pieces were removed from the tubes and flushed with saline. Each catheter piece was then gently agitated in three consecutive saline baths. The purpose of the rinsing procedure was to remove residual urine and loosely attached organisms from the surface of the catheters. Three 1-cm segments were cut from each catheter. Each 1-cm segment was placed in 1 mL of saline and sonicated at 55,000 Hz for 10 minutes in a water bath sonicator (Buehler Scientific, Evanston, Ill) at room temperature.¹⁷ The sonication fluid was diluted to $1:10^3$, and 100 μ L aliquots of the original and diluted sonication fluid were inoculated onto agar plates to determine the plate counts. Gram staining of selected colonies was done to verify the types of organisms. Samples of the urine in each centrifuge tube were also diluted and spread on agar plates to assess the final bacterial concentrations and purity. For each tested uropathogen, this set of experiments described above was repeated up to nine times.

Statistical Analysis

For the purposes of statistical comparison, the median of the colony counts from the 1-cm catheter segments was used as the value for each catheter. The Wilcoxon two-tailed signed rank test was used for all comparisons using Statistical Analysis System software.

RESULTS

The pre-existing coating of *E. coli* 83972 significantly reduced catheter colonization by all tested uropathogens (Fig. 1). *P. stuartii*-alone catheters had a median of 3.6×10^3 cfu/cm of *P. stuartii*, and the *E. coli* plus *P. stuartii* catheters had a smaller median of 1.9×10^2 cfu/cm of *P. stuartii* ($P=0.03$, Wilcoxon signed rank test). For the uropathogenic lactose-negative *E. coli* catheters, 1.11×10^4 cfu/cm were found on the catheter surface when the catheter had not been previously exposed to *E. coli* 83972 versus only 8.5×10^1 cfu/cm when the catheter had been pre-exposed to *E. coli* 83972 ($P = 0.03$). For *C. albicans* catheters, 2.8×10^3 cfu/cm and 10 cfu/cm were found in the absence and presence of *E. coli* 83972, respectively ($P = 0.02$). In the cases of *P. stuartii* and lactose-negative *E. coli*, preinoculation of the catheters with *E.*

coli 83972 reduced the concentration of uropathogens on the catheter surface in all six sets of experiments. A similar observation was noted in eight of nine sets of experiments with *C. albicans*. In no case did incubation with uropathogens significantly reduce the number of *E. coli* 83972 recovered from the catheter surface.

COMMENT

Catheter-related UTI can arise when a few organisms gain entry to the urinary tract and then multiply to high concentrations in the bladder.^{18,19} The results of this in vitro study suggest that a urinary catheter with a pre-existing coating of *E. coli* 83972 is likely to be effective at inhibiting even a relatively large number of pathogenic organisms belonging to multiple different genera from colonizing the catheter and thus gaining access to the bladder. Two aspects of these results are particularly encouraging. First, *E. coli* 83972 protected against catheter colonization by a variety of uropathogens, including gram-positive,¹³ gram-negative, and fungal uropathogens, and second, exposure of the *E. coli* 83972-coated catheters to uropathogens did not diminish the numbers of *E. coli* 83972 recovered from the catheter surface.

The potentially broad-spectrum protection afforded by *E. coli* 83972 is particularly important, because it may help avoid the common clinical scenario in which suppression of one species of uropathogen allows other strains to flourish. Indeed, the failure to suppress all strains of uropathogens is the major pitfall to the long-term use of antimicrobial agents for UTI prevention in chronically catheterized patients. When prophylactic antibiotics are given to patients with a neurogenic bladder and chronic bacteriuria, the colonizing flora rapidly shift from antibiotic-susceptible organisms to antibiotic-resistant organisms.^{20,21} Using a broadly interfering agent such as *E. coli* 83972 to prevent UTI may avoid this problem of supercolonization with resistant flora. Another potential benefit of using bacterial interference rather than antimicrobial agents for UTI prevention may be that patients take fewer courses of antibiotics and thus experience fewer adverse drug effects. Our bacterial interference approach to UTI prevention is designed to decrease the use of antimicrobial agents and their attendant complications.

We found that recovery of *E. coli* 83972 from the catheters was not significantly affected by the presence or absence of uropathogens in the urine. The implication of this finding is that *E. coli* 83972 may persist on the surface of urinary catheters when inserted into patients, whose bladders may contain multiple species of uropathogens. If catheter colonization with *E. coli* 83972 proves to be durable in vivo, the *E. coli* 83972 on the catheter surface may serve as a nidus to continually seed the bladder with this protective strain. Currently, a pilot clinical trial is underway to examine the safety and efficacy of placing urinary catheters with a pre-existing coating of *E. coli* 83972 in SCI patients with a neurogenic bladder and recurrent UTI.

E. coli 83972 may have several advantages as an agent of bacterial interference for prevention of UTI compared with *Lactobacillus*, an organism that has been extensively studied. In vitro, various species of *Lactobacillus* can block adherence of uropathogens to vaginal epithelial cells, secrete antibacterial agents, and even produce an antifungal compound.^{22–24} In vivo, deliberate intravaginal instillation of *Lactobacillus casei* GR-1 into 5 women with recurrent UTI reduced colonization of the urogenital epithelium by coliforms but not by enterococci.²⁵ A subsequent study in 41 adult women with recurrent UTI suggested that giving intravaginal *Lactobacillus* suppositories after completion of antibiotic therapy helped restore the indigenous vaginal flora and reduced the recurrence rate of UTI.²⁶ Although the work with *Lactobacillus* is promising, *Lactobacillus* does not colonize the bladder and is found exclusively on the female urogenital epithelium.^{26,27} Therefore, this approach using *Lactobacillus* may not be applicable to men or to patients with indwelling catheters. Because

our target population is individuals with a neurogenic bladder secondary to SCI, many of whom are male and require indwelling catheters, *E. coli* 83972 is probably a better agent of bacterial interference for our purposes.

The mechanisms underlying the interference effects of *E. coli* 83972 are not known. Possible mechanisms include spatial constraints, competition for locally available nutrients, and secretion of inhibitory agents by *E. coli* 83972.²⁸ Because the effect of bacterial interference manifests on the catheter surface, the characteristics of the biofilm formed by *E. coli* 83972 likely play a central role in its ability to act as an agent of bacterial interference. Elucidating the molecular mechanism of growth inhibition of uropathogens by *E. coli* 83972 will be a subject of future experiments.

We also do not know whether the observed decrease in the numbers of uropathogens attached to catheter surfaces will translate in vivo to decreased numbers of free-floating uropathogens in patients' urine. One of the aims of the planned in vivo trial of *E. coli* 83972-coated catheters will be to compare the numbers and types of organisms recovered from catheter surfaces to the numbers and types of organisms present in the urine.

CONCLUSIONS

The results of this in vitro study demonstrated that a pre-existing coating of *E. coli* 83972 on the surface of urinary catheters significantly impedes colonization by a variety of uropathogens. This promising approach to bacterial interference invites additional clinical investigation.

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FIGURE 1.

E. coli 83972 inhibits catheter colonization by uropathogens. Data from six to nine sets of experiments for each tested uropathogen are represented. Gram-negative and fungal uropathogens were significantly less likely to colonize urinary catheters with a pre-existing coating of *E. coli* 83972 than control catheters without *E. coli* 83972.