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PRE-INOCULATION OF URINARY CATHETERS WITH ESCHERICHIA COLI 83972 INHIBITS CATHETER COLONIZATION BY ENTEROCOCCUS FAECALIS

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Abstract

Purpose—The capacity of a preexisting coating of *Escherichia coli* 83972 to reduce catheter colonization by *Enterococcus faecalis* 210 was investigated. *Enterococcus* was chosen for these trials since it is a common urinary pathogen in patients with an indwelling urinary catheter.

Materials and Methods—Each experiment tested 3 growth conditions. Group 1 or *E. coli* plus *Enterococcus* catheters were exposed to *E. coli* 83972 for 24 hours and then to *Enterococcus* for 30 minutes. Group 2 or *E. coli* alone catheters were incubated in *E. coli* for 24 hours and then in sterile broth for 30 minutes. Group 3 or *Enterococcus* alone catheters did not undergo the initial incubation with *E. coli* before the 30-minute incubation with *Enterococcus*: All catheters were then incubated in sterile human urine for 24 hours. Catheters were washed with saline and cut into 5, 1 cm. segments. Each segment was sonicated and the sonication fluid was diluted and plated. The results of each of the 5 segments were averaged and the set of experiments was repeated 7 times.

Results—A preexisting coating of *E. coli* 83972 reduced catheter colonization by *E. faecalis* 210 more than 10-fold. *Enterococcus* alone catheters had a median of 9.7×10^5 enterococci per cm., whereas *E. coli* plus *Enterococcus* catheters had a median of 0.38×10^5 enterococci per cm. ($p = 0.016$).

Conclusions—Pre-inoculating urinary catheters with *E. coli* 83972 significantly impedes catheter colonization by *Enterococcus*: These promising in vitro results prompt the clinical investigation of this particular application of bacterial interference.

Keywords

urinary tract; urinary tract infections; *Escherichia coli*; catheters; indwelling; antibiotic

Urinary tract infection is the most common infection in patients with a chronic indwelling bladder catheter.^{1, 2} Although the closed catheter system delays bacterial entry into the urinary tract, bacteriuria still develops in the majority of patients within 2 weeks of continuous catheter use.^{3, 4} Essentially all patients with an indwelling urinary catheter for 30 days or less have bacteriuria, commonly with more than 1 organism.^{1, 5, 6} In many cases this bacteriuria is asymptomatic but when catheter associated urinary tract infection develops, the infection is often difficult to treat.⁷ In the absence of symptoms bacteria in the bladder are sometimes considered benign colonizers and often remain untreated.

The concept of using nonpathogenic bacteria for preventing symptomatic infection is known as bacterial interference.^{8, 9} The finding of Lindberg et al that asymptomatic bacteriuria in girls

did not cause renal damage¹⁰ was the first step toward using bacterial interference for preventing symptomatic urinary tract infection. Subsequently studies revealed that treating asymptomatic bacteriuria in girls led to a change in urinary flora and would increase the risk of acute pyelonephritis.¹¹ Studies in elderly subjects, patients with spinal cord injury and women indicate that treating asymptomatic bacteriuria in these populations is neither beneficial nor effective.^{12–15} Allowing asymptomatic bladder colonization to persist to prevent colonization by a pathogenic strain may be a passive approach to bacterial interference.¹⁴

A more active approach to bacterial interference involves intentionally colonizing the bladder with a nonpathogenic organism to prevent infection by more virulent bacteria. In a prospective nonrandomized trial inducing deliberate bladder colonization by the nonpathogenic *Escherichia coli* 83972 strain in patients with spinal cord injury and neurogenic bladder significantly reduced the rate of symptomatic urinary tract infection.¹⁶ Of 44 spinal cord injured patients who achieved bladder colonization longer than 1 month in duration after deliberate bladder inoculation with *E. coli* 83972, 30 experienced a 50-fold reduction in the rate of symptomatic urinary tract infection compared with the baseline rate of urinary tract infection before the study period.¹⁶ However, the major limitation of this bladder inoculation study was the difficulty of establishing long-term colonization of this organism. Of 93 inoculation attempts only 47 (51%) were successful. Also, *E. coli* 83972 often cleared spontaneously from the bladder. Pre-inoculating urinary catheters with *E. coli* 83972 before placing them may enhance the protective effect of bacterial interference by 2 means. The persistence of *E. coli* 83972 in the bladder may be improved and the established presence of this nonpathogenic strain of *E. coli* may reduce the likelihood of pathogenic organisms establishing catheter colonization, bladder colonization and perhaps symptomatic urinary tract infection. With this goal in mind we investigated whether previous inoculation of urinary catheters with *E. coli* 83972 would reduce catheter colonization by *Enterococcus faecalis* in vitro. *Enterococcus* was chosen because enterococci are a common cause of urinary tract infection in patients with a chronic indwelling catheter.⁴ In addition, the increasing prevalence of antibiotic resistant enterococci suggests that a nonpharmaceutical approach to the control of this pathogen would be useful.¹⁷

MATERIALS AND METHODS

Bacteria

E. coli 83972, serotype ON:KN (N = nontypable) was initially isolated from the urine of a girl with a 3-year history of asymptomatic bacteriuria with stable renal function.¹⁸ This strain was selected because of its documented ability to persist in the urinary tract without causing disease. *E. faecalis* 210 was initially isolated from the urine of a patient with catheter related urinary tract infection. Each strain was stored at –80C in 10% glycerol culture and then passaged on trypticase soy agar II with 5% sheep blood (BBL, Cockeysville, Maryland). Just before performing the catheter studies bacteria were retrieved from the trypticase soy agar plates and cultured overnight in trypticase soy broth (Difco, Sparks, Maryland). Bacteria were harvested by centrifugation and re-suspended in sterile saline to a concentration of 10⁸ colony-forming units per ml.

Experimental variables and controls

Each experiment tested 3 growth conditions. Group 1 or *E. coli* plus *Enterococcus* catheters were exposed to *E. coli* 83972 for 24 hours and then to *Enterococcus* for 30 minutes. Group 2 or *E. coli* alone catheters were incubated in *E. coli* for 24 hours and then in sterile broth for 30 minutes. Group 3 or *Enterococcus* alone catheters did not undergo the initial incubation with *E. coli* before the 30-minute incubation with *Enterococcus* (fig. 1).

Catheter preparation

Foley catheters used in this experiment were composed of latex with a hydrophilic coating (Bardex, Covington, Georgia). Pilot trials had demonstrated optimal adherence of *E. coli* 83972 to this catheter material compared with silicone (data not shown). All catheters were 12Fr with a 5 cc ribbed balloon. Under sterile conditions each end of each catheter was removed and discarded. The remaining catheter shaft was cut into 8 cm. pieces. The balloon lumen of each piece was plugged at the 2 ends with sterile pipette tips and sealed with flame, leaving only the main catheter lumen open. The catheter pieces were placed into sterile 50 cc centrifuge tubes containing trypticase soy broth.

Bacterial interference trial

For growth condition 1 catheter pieces were placed into sterile 50 ml. centrifuge tubes containing trypticase soy broth. *E. coli* 83972 was added to each tube at a starting concentration of 10^5 colony-forming units per ml. The catheter pieces were incubated for 24 hours at 37C in the *E. coli* suspension. Catheter pieces previously exposed to *E. coli* 83972 were next placed in a suspension of 10^4 colony-forming units per ml. *E. faecalis* in trypticase soy broth at 37C without washing. After 30 minutes of incubation in the *Enterococcus* suspension the catheter pieces were removed and placed immediately in filter sterilized human urine at 37C. The catheters were incubated in urine at 37C for 24 hours (fig. 1).

Under growth condition 2 the initial 24-hour incubation in *E. coli* 83972 was done according to the same procedure but the broth in the 30-minute incubation step was sterile. In contrast, group 3 catheters remained untouched during the initial 24-hour incubation of groups 2 and 3 catheters, and during the 30-minute incubation step they were placed in trypticase soy broth containing 10^4 per ml. *E. faecalis*. All groups 1 to 3 catheters were transferred to sterile urine after the 30 minutes incubation step and incubated for 24 hours at 37C. Urine was obtained from a single male donor and filter sterilized by passage through a 0.22 μ m. filter.

Catheter harvest

Under sterile conditions the 8 cm. catheter pieces were removed from the tubes and flushed with 60 cc sterile saline using a needle and syringe. The 2 ends of each piece were cut off and the balloon lumen was flushed with 30 cc saline. The catheter was then gently agitated in 3 separate saline baths. Each lumen was rapidly flushed with air. Five 1-cm. segments were cut from each catheter. Each 1 cm. segment was placed in 1 cc saline and sonicated at 55,000 Hz. for 10 minutes in a water bath sonicator (Buehler Scientific, Evanston, Illinois).¹⁹ Sonication fluid was diluted to 1:10², and 100 μ l. aliquots of original and diluted sonication fluid were inoculated onto blood and colistin-nalidixic acid agar plates to determine plate counts. Gram staining was done to verify the types of organisms recovered. A sample of the urine in each centrifuge tube was also diluted and plated to assess final bacterial concentration and purity.

Statistical analysis

Data on the 5, 1 cm. segments were averaged to determine the mean colony-forming units per cm. per catheter. Each set of experiments was repeated 7 times. The Wilcoxon 2-tailed signed rank test was done for all comparisons using commercially available software.

RESULTS

The preexisting coating of *E. coli* 83972 reduced catheter colonization by *E. faecalis* 210 by a more than 10-fold difference (figs. 2 and 3). *Enterococcus* alone catheters had a median of 9.7×10^5 enterococci per cm., whereas *E. coli* plus *Enterococcus* catheters had a median of 0.38×10^5 enterococci per cm. ($p = 0.016$). The number of *E. coli* found on the catheters after

incubation for 24 hours in urine was similar regardless of the presence or absence of *Enterococcus* (median 6.7×10^4 versus 9.9×10^4 colony-forming units *E. coli* per cm., $p = 0.47$, fig. 4).

We also compared the final concentrations of *E. coli* 83972 and *E. faecalis* 210 in the urine. In tubes containing *E. coli* plus *Enterococcus* catheters the median concentration of *E. coli* 83972 was 1.2×10^8 colony-forming units per ml. urine, while the median concentration of *E. coli* 83972 in tubes containing *E. coli* alone catheters was 1.1×10^8 colony-forming units per ml. ($p = 1.0$, fig. 5). These results indicate that *E. faecalis* did not inhibit the growth of *E. coli* 83972 in urine.

However, the presence of *E. coli* in the urine appeared to inhibit enterococcal growth in urine, although this trend did not reach statistical significance. Tubes containing *Enterococcus* alone catheters had a median concentration of 5.3×10^7 enterococci per ml. urine, while tubes with *E. coli* plus *Enterococcus* catheters had a median concentration of 0.86×10^7 enterococci per ml. urine ($p = 0.063$, fig. 6).

DISCUSSION

The greater than 10-fold reduction in catheter colonization by *E. faecalis* 210 when the catheter had a pre-existing coating of *E. coli* 83972 indicates that *E. coli* 83972 can effectively interfere with enterococcal growth on a urinary catheter. Although in these experiments we exposed *E. coli* coated catheters to a relatively high concentration of enterococci (1×10^4 colony-forming units per ml.), *E. coli* still significantly impeded colonization by enterococci. In addition, the presence of *Enterococcus* did not affect the concentration of *E. coli* on the catheter surface. Thus, *Enterococcus* did not appear to replace *E. coli* on the catheter surface, at least during the 24-hour incubation period. Catheter related urinary tract infection may arise when a small number of organisms gain entry and then multiply to high concentrations in the bladder.^{2, 20} Our results suggest that a urinary catheter with a preexisting coating of *E. coli* is likely to be effective for preventing a small number of pathogenic organisms from colonizing the catheter and the bladder, eventually causing urinary tract infection.

Our preliminary results also indicate that *E. coli* 83972 inhibits growth of *E. faecalis* 210 in urine. However, the presence of *E. faecalis* did not affect the growth rate of *E. coli* 83972 in urine. These findings prompted us to study further the ability of *E. coli* 83972 to inhibit enterococcal growth. *E. coli* 83972 was inoculated with a stab into solid medium, grown overnight, killed with chloroform and then overlaid with a soft agar containing *E. faecalis* 210.²¹ Using this stab overlay method we found that killed *E. coli* 83972 inhibited the growth of *E. faecalis* 210 in a zone around the stab. These observations indicate that *E. coli* 83972 synthesizes a factor or factors that inhibit the growth of *E. faecalis*. Elucidating the molecular mechanism of the growth inhibition of *Enterococcus* by *E. coli* 83972 is planned to be a subject of our future experiments.

Although to our knowledge the mechanism of the inhibition of *Enterococcus* by *E. coli* is not currently known, the inhibitory effect of *Lactobacillus* on *Enterococcus* has been well studied. In a parallel plate flow chamber *Lactobacillus* was able to displace adherent *E. faecalis* from hydrophobic and hydrophilic substrata.²² Many strains of *Lactobacillus* produce biosurfactants or microbial products that affect the surface tension at liquid-air interfaces.²³ Several isolated biosurfactants were able to slow the initial deposition rates and decrease the number of adherent cells of uropathogenic *E. faecalis* to hydrophilic glass and to hydrophobic silicone rubber in a parallel plate flow chamber.^{23–25} Molecular analysis of the biosurfactant produced by lactobacilli revealed that most of the anti-enterococcal activity was attributable to a 29 kDa. protein that is thought to act as an adhesin.²⁶ Therefore, the same proteins that help lactobacilli

bind to host components, such as the extracellular matrix, may also help to exclude binding by pathogenic organisms.

While these in vitro studies with *Lactobacillus* suggest that the scientific mechanisms underlying bacterial interference can be elucidated, *Lactobacillus* has several limitations as an agent of bacterial interference for preventing urinary tract infection in vivo. *Lactobacillus* does not colonize the bladder and is found exclusively on the female urogenital epithelium.^{27, 28} Thus, *Lactobacillus* may not be an effective agent of bacterial interference in men or in patients with an indwelling catheter.

E. coli 83927 may have broader applicability as an agent of bacterial interference for preventing urinary tract infection. In prospective nonrandomized human studies bladder inoculation with *E. coli* 83972 has proved to be safe and effective for protecting against symptomatic urinary tract infection.^{16, 29} We anticipate that inserting bladder catheters with pre-formed coating of *E. coli* 83972 may be more successful than direct inoculation for establishing and maintaining bladder colonization with *E. coli* 83972.

This particular approach to bacterial interference using urinary catheters pre-inoculated with *E. coli* 83972 is designed to avoid the use of antibiotics and their attendant complications. Clinical trials of antimicrobial agents for irrigating the catheter or bladder,^{30, 31} disinfecting the collection bag^{32, 33} or cleansing the urethral meatus^{34, 35} have yielded disappointing results. Antibiotics can suppress the development of bacteriuria in patients with short-term indwelling catheters but in those who are chronically catheterized antibiotics favor the development of highly resistant urinary flora.^{1, 4} Furthermore, using antibiotics in all patients with a urinary catheter would increase the incidence of adverse drug reactions and supra-infections, such as *Clostridium difficile* colitis.¹ Since catheter associated urinary tract infection is a major cause of morbidity and mortality in patients with an indwelling catheter,^{1, 2} a new approach for preventing urinary tract infection in this population is urgently needed.

The encouraging results of this initial in vitro study invite further investigations in vitro and in vivo. In vitro the ability of a pre-formed biofilm of *E. coli* 83972 to prevent catheter colonization by bacterial genera other than *Enterococcus* merits investigation. In vivo a pilot clinical trial is planned to examine the safety and efficacy of placing urinary catheters with a preexisting coating of *E. coli* 83972 in patients with neurogenic bladder and recurrent urinary tract infection. If pilot trials of this approach to bacterial interference are successful in the spinal cord injured population, the trials can be extended to other catheter dependent populations, including geriatric patients and nursing home residents.

CONCLUSIONS

This in vitro study demonstrated that a pre-existing coating of *E. coli* 83972 on the surface of urinary catheters significantly impeded colonization by *Enterococcus faecalis* 210. This promising approach to bacterial interference invites further clinical investigation.

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Fig. 1.
Experimental protocol. *TSB*, trypticase soy broth

Fig. 2.

Catheters pre-inoculated with *E. coli* 83972 had significantly fewer enterococci on surfaces than those without previous exposure to *E. coli* ($p = 0.016$). Each point represents data from 1 of 7 trials, indicating average enterococci per ml. sonicate fluid recovered from 5, 1 cm. segments cut from single catheter. *CFU*, colony-forming units.

Fig. 3. Slightly overgrown *E. faecalis* 210 showing dramatic reduction in number of enterococcal colonies retrieved from catheters pre-inoculated with *E. coli* 83972. A, colistin-naladixic acid plate with fewer colonies received 100 μ l. sonicate fluid from 1 cm. segment of *E. coli* plus Enterococcus catheter. Colistin-naladixic acid was chosen to eliminate *E. coli*. B, blood agar plate with more colonies received 100 μ l. sonicate fluid from 1 cm. segment of Enterococcus alone catheter. Enterococcal colony counts did not differ in blood agar and colistin-naladixic acid plates.

Fig. 4.

Exposure of *E. coli* coated catheters to *Enterococcus* did not significantly affect number of *E. coli* recovered from catheters ($p = 0.47$). Each point represents data from 1 of 7 trials, indicating average *E. coli* per ml. sonicate fluid recovered from 5, 1 cm. segments cut from single catheter. *CFU*, colony-forming units.

Fig. 5.

Whether catheter was exposed to Enterococcus before placement in urine did not affect final concentration of *E. coli* in urine ($p = 1$). Each point represents data from 1 of 7 trials, indicating colony-forming units (CFU) of *E. coli* per ml. urine after catheter incubation in urine for 24 hours.

Fig. 6.

When *E. coli* 83972 was present on catheter surface when catheter was placed in urine, fewer enterococci were recovered from urine 24 hours later. This trend did not reach statistical significance because only 5 of 7 trials provided reliable results of final urinary Enterococcus concentration ($p = 0.063$). Each point represents data from 1 of 5 trials, indicating colony-forming units (CFU) of *E. faecalis* per ml. urine after catheter incubation in urine for 24 hours.