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PREFERENTIAL ADHESION OF URETHRAL BACTERIA FROM A MIXED POPULATION TO A URINARY CATHETER

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Abstract

The ability of uropathogens to adhere to catheters and subsequently colonize the urinary mucosa leads to urinary tract infections which afflict a large patient population. *In vitro* studies were carried out whereby *Escherichia coli* Hu734 (water contact angle 12°) and *Enterococcus faecalis* (19°) were found to be highly adhesive to silicone latex urinary catheters. The addition of one of four *Lactobacillus* sp., with water contact angles ranging from 19-105°, to the suspending fluid caused a 60-86% reduction in pathogen adhesion with a significant effect against *E. coli*, the organism most commonly found to infect the urinary tract. Lactobacilli were significantly effective at displacing uropathogens and preventing their adhesion. Hydrophobic lactobacilli (105°) were particularly effective at preventing enterococci from adhering from the surface, while more hydrophilic lactobacilli (19-54°) were most effective at displacing enterococci. The effective competition with four strains of lactobacilli was achieved even when they only comprised 0.1%-7% of the total organisms on the surfaces. These studies demonstrate the important role which the indigenous urethral flora could play in inhibiting the initial attachment of pathogens to catheter surfaces.

Key Words: Urethral bacteria, preferential adhesion, urinary catheter, lactobacillus competition.

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Introduction

When a catheter is implanted into the bladder through the urethra, the surface of the prosthesis immediately comes into contact with urinary suspending fluid, mucosal tissue and bacteria adherent to that tissue (Reid *et al.*, 1992f, 1992g). If the device is implanted without antimicrobial coverage, colonization, asymptomatic bacteriuria and urinary tract infection (UTI) can arise within the first 48 hours (Kunin, 1987). This occurs when the uropathogenic bacteria, most commonly *Escherichia coli* and *Enterococcus faecalis*, adhere and form biofilms on the catheter surface, then seed the bladder mucosa where multiplication leads to further formation of drug resistant biofilms and symptomatic and asymptomatic disease (Nickel *et al.*, 1985; 1989; Costerton *et al.*, 1987; Reid *et al.*, 1992b; 1992d; 1992e; 1993b).

Antibiotic treatment of well-formed biofilms is invariably ineffective, while prophylaxis may kill approaching organisms and prevent infection over a few days (Costerton *et al.*, 1987). Recent *in vitro* studies have suggested that potent agents such as ciprofloxacin, can prevent, to a large extent, biofilm formation. In effect, "young" (less than 24 hour) biofilms could be eradicated from catheters with ciprofloxacin (Reid *et al.*, 1993b). Previous studies have shown that antimicrobial therapy can disrupt the normal urethral flora, making the patient more susceptible to recurrent UTI (Reid *et al.*, 1990a, 1992a). The question raised is does this eradication of indigenous flora remove organisms which could help protect the host from infection?

Extensive studies have shown that the presence of certain strains of *Lactobacillus*, normally the dominant organism on the female urethra, can exclude uropathogens from adhering to uroepithelial cells (Chan *et al.*, 1984; Reid *et al.*, 1987) and polymer substrata (Hawthorn and Reid, 1990a), as well as prevent their growth (Reid *et al.*, 1988; McGroarty and Reid, 1988a; 1988b). These studies have led to the use of lactobacilli to effectively reduce UTI rates in animals (Reid *et al.*, 1985) and humans (Bruce and Reid, 1988; Bruce *et al.*, 1992; Reid *et al.*, 1992a). The possibility that lactobacilli could reduce the adhesion of uropathogens to urethral catheters provided the impetus for the present study.

Materials and Methods

Catheters

One centimeter sections (inner diameter 3 mm) of silicone latex urethral catheters were provided by Rusch-Pilling Inc., Canada and were used in this study.

Bacterial adhesion assay

Two strains of uropathogens, *Escherichia coli* Hu 734 and *Enterococcus faecalis* 296 were cultured separately overnight in brain heart infusion yeast extract broth (Difco, Detroit). *Lactobacillus fermentum* B-54, *L. acidophilus* T-13 and 76, and *L. casei* 36 were cultured separately overnight in MRS broth (Difco). Concentrations of approximately 1 x 10^8 organisms per ml suspending fluids were incubated with the catheter sections for 1 hour in a 37°C rotary bath. In the displacement experiments, the pathogens were incubated for 1 hour with the catheters prior to addition of the lactobacilli for a further hour. In the precoating experiments, the lactobacilli were incubated first, then challenged with the pathogens. For the preferential adhesion tests, all three species (one lactobacillus and both pathogens) were incubated with the catheter sections for one hour. Each experimental run used one catheter piece. All reported results represent triplicate data. The one hour duration for the adhesion studies was selected as we were interested in the early part of the infection process and as preliminary experiments had shown that the two uropathogens adhered to the catheters equally well after one and twenty four hours (*E. coli* 8.6 x 10^4 after 1 hour, 2.8 x 10^5 after 24 hours; *E. faecalis* 2.63 x 10^4 and 1.2 x 10^4).

The suspending fluid in all cases was phosphate buffered saline (PBS), pH 7.1, rather than urine, as the latter would have interfered with reproducibility, growth and adhesion (Hawthorn and Reid, 1990b). After incubation, the catheter sections were subjected to washing in PBS. Adherent bacteria were removed for viable plate counting by sonicating in an ultrasonic waterbath for 5 minutes. The number of viable bacteria was recorded. The different selective media used to differentiate organisms was Rogosa agar (Difco) for the *Lactobacillus* sp, MSA agar (Difco) for enterococci and MacConkeys agar (Difco) for *E. coli*.

Water contact angle measurements

The water contact angle technique was used to determine the relative hydrophobicity of the organisms. This has been described originally by Van Oss and Gillman (1972) and more recently by van der Mei *et al.* (1991) and Reid *et al.* (1992c). In brief, bacterial cells were harvested and washed in Millipore Q water by centrifugation at 10,000 x g for 10 minutes. Bacterial lawns were prepared on cellulose acetate membrane filters by negative pressure filtration, and the filters were glued to a thin layer of dental wax just above its melting point on an aluminium disc and immediately fixed by placing in contact with ice. Discs with mounted filters were dried at 37°C for 2-3 hours. Contact angles were measured using two droplets of water per filter.

Results

Table 1 demonstrates the hydrophobicity of the organisms and their adhesive capacity to the catheter material after one hour incubation. The hydrophilic uropathogens were the most adherent of all the strains tested. The water contact angle of the catheter could not be obtained with compete accuracy, due to the roundness of the material. However, it would appear to be hydrophobic based upon its chemistry. When the two uropathogens were coincubated with the catheter, 111,800 bacteria adhered per cm section (Figure 1). This was then referred to as 100% coverage, and all subsequent data were compared to this value. The standard deviations for all the results fell within the 20% level, and only the mean results are presented on the graphs. However, for statistical evaluations, all the raw data were analyzed by one way analysis of variance and Dunnett's test. This showed a significant reduction in *E. coli* adhesion with all four lactobacilli in every experiment ($p < 0.01$). As Figure 1 shows, there was significant (> 82%) ($p = 0.0001$) displacement of both *E. coli* and *E. faecalis* adhesion when lactobacilli were used to treat the device. However, analysis of each pathogen showed that the levels were not significant for hydrophobic lactobacilli (B-54 and T-13) against enterococci. The data did demonstrate that after the experiments, strain 76 only comprised 0.1% of all the adherent organisms, yet the numbers of pathogens had been significantly reduced. When *E. coli* alone was challenged by lactobacilli, there was an overall 87% displacement (82% for B-54, 83% T-13, 98% 76, 86% 36), compared to 97% for enterococci (100% B-54, 95% T-13, 93% 76, 100% 36).

As Figure 2 shows, precoating the surfaces with lactobacilli significantly (> 80%)($p = 0.0001$) reduced the number of uropathogens subsequently able to adhere. There was a higher proportion of hydrophobic lactobacilli present amongst the flora on completion of the experiments, and this reflected in significant blockage of enterococci with strain B-54 ($p < 0.01$), but not the other three lactobacilli. In effect, the uropathogens displaced 99% of strains 76 and 36, yet the pathogens were still not able to adhere in the numbers found for uncoated devices. When the challenge was carried out with *E. coli* alone, there was a mean of 90% fewer pathogens adhered compared to controls, and for *E. faecalis* there were 95% fewer pathogens.

When lactobacilli were present in the suspending microbial milieu in conjunction with *E. coli* and *E. faecalis*, there was a net reduction in uropathogenic adhesion of 60-86% ($p = 0.0001$) (Figure 3). This procedure especially reduced the *E. coli* adhesion (from 75% of the organisms on a 1 cm section to 1.9-6%)($p = 0.0001$ overall, $p < 0.01$ for each comparison). However, there was no significant reduction amongst the enterococci adherent within the mixtures, and in fact the proportion of adherent enterococci actually increased in the presence of *L. acidophilus* T-13 and 76.

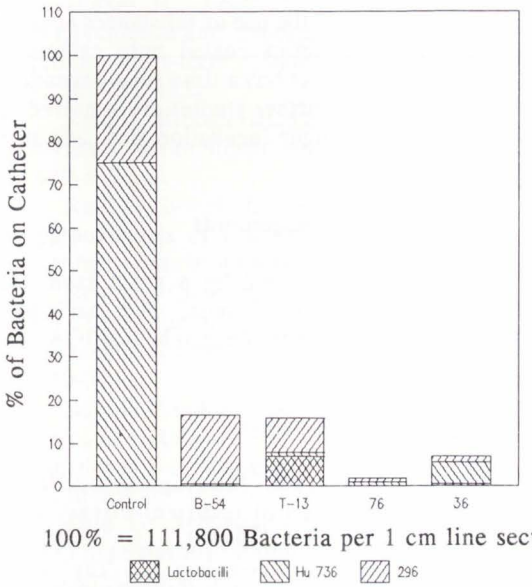

Figure 1. Incubation of urinary catheter with jointly *E. coli* Hu734 and *E. faecalis* 296, then challenged for 1 hour with *L. fermentum* B-54, *L. acidophilus* T-13, *L. acidophilus* 76, and *L. casei* 36. Results are presented in percentage as relation to control.

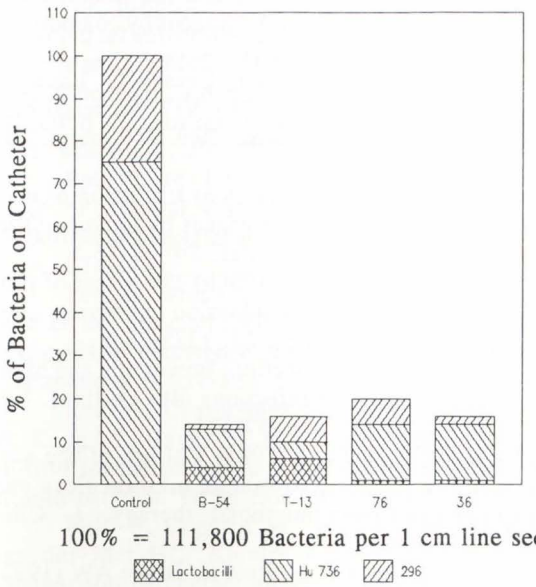

Figure 2. Precoating of urinary catheters for 1 hour with lactobacilli followed by challenge jointly with *E. coli* Hu734 and *E. faecalis* 296. Results are presented in percentage as relation to control.

Discussion

It has been well documented that uropathogens can be highly adherent to uroepithelial cells (Reid and Brooks, 1984) and to polymer materials (Reid *et al.*,

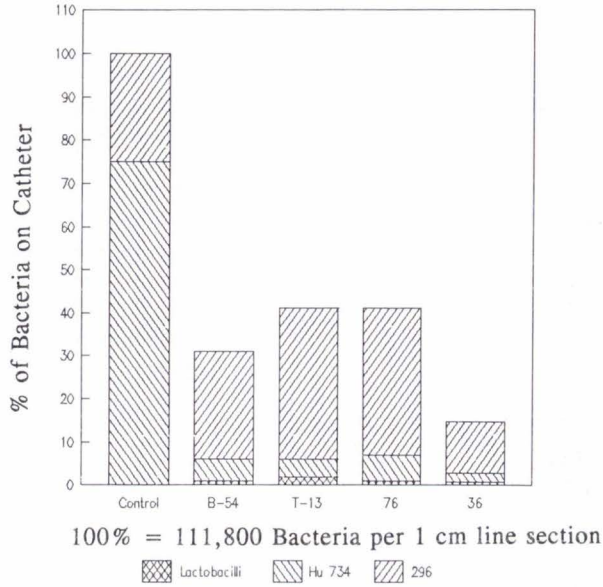

Figure 3. Co-incubation of three organisms with urinary catheters for 1 hour. Results are presented in percentage as relation to control.

Table 1. Adhesion of urethral organisms to silicone latex urinary catheter

Strain	Water Contact Angle	Adhesion*
L.fermentum B-54	105	$1.4 \pm 0.2 \times 10^4$
L.acidophilus T-13	80	$4.2 \pm 0.4 \times 10^2$
L.acidophilus 76	54	$3.5 \pm 0.5 \times 10^3$
L.casei 36	19	$4.4 \pm 0.2 \times 10^4$
Enterococcus faecalis 1131	19	$4.0 \pm 0.8 \times 10^6$
Escherichia coli Hu734	12	$1.0 \pm 0.1 \times 10^5$

*Number of viable bacteria adherent to a 1 cm section of catheter. Experiments performed in triplicate.

1989). It has also been shown that preincubation of polymers with *L. acidophilus* T-13 can block the subsequent adhesion of *E. coli* and *Staphylococcus epidermidis* (Hawthorn and Reid, 1990a). The latest findings verify that this competitive exclusion effect can occur against the same *E. coli* and another Gram positive strain, namely of *E. faecalis*, on commercially available urinary catheters. This was a particularly significant finding, as the *E. coli* strain adhered much better to the catheters than to individual polymers (Reid *et al.*, 1991), thus the exclusion found here had to be highly effective. It is also important to point out that the hydrophilic *E. coli* adhered well to the hydrophobic surface of the catheter, implying that forces other than hydrophobic ones (perhaps electrostatic forces or deposition of bacterial

byproducts) were responsible for adhesion. One explanation for the differences between polymer and catheter results is that the surface of a commercial catheter is not as evenly shaped and as universally hydrophobic as an individual polymer such as fluorinated ethylene propylene (Reid *et al.*, 1989).

The ability of the lactobacilli to actually displace adherent pathogens from catheters has not been reported previously. The displacement of enterococci was most effective when they were the sole colonizers, rather than when they coexisted with *E. coli*. The 94% displacement of type 1 and P fimbriated *E. coli* was particularly significant as these organisms are the most virulent found in the urinary tract, causing bladder and kidney infections (Reid and Sobel, 1987). It is not clear to what extent hydrophilic or hydrophobic components on lactobacilli actually were responsible for any displacement, nor what caused the difference between *E. coli* and enterococcal displacement. It is possible that coaggregation occurred between the species, whereby the binding of lactobacilli to the pathogens resulted in detachment (Reid *et al.*, 1990b), as well as in coadhesion of enterococci and lactobacilli (McGroarty *et al.*, 1992). In addition, or as an alternative, the forces of surface tension may well have played a role in the displacement. A previous study showed that *S. epidermidis* and *E. coli* appeared to produce factors which altered the suspending fluid in such a way that lactobacilli adhesion increased (Hawthorn and Reid, 1990a). It could also be hypothesized that lactobacilli deposited substances onto the catheter, which then inhibited subsequent adhesion by pathogens. The existence of extracellular adhesins on lactobacilli has been recently reported (Reid *et al.*, 1993a), but their effect on other organisms is unclear.

More than one species of bacteria invariably colonize urinary catheters (Kunin and Steele, 1985) and different species can cause bacteriuria (Schaeffer, 1986). The results here showed that Gram positive and Gram negative pathogens can bind to catheters and coexist in large numbers on prosthetic device surfaces. The possibility that non-pathogenic indigenous flora might compete with pathogens and reduce the incidence of UTI seems feasible, and evidence exists from human mucosal studies to support this concept (Bruce and Reid, 1988; Reid *et al.*, 1992a; G. Reid, A. W. Bruce, and M. Taylor. Supplementation of the vaginal flora with lactobacillus and stimulation of indigenous organisms to prevent recurrence of urinary tract infections: a randomized clinical trial. Submitted to J. Urol.). In the present study, the fact that the inclusion of lactobacilli with two uropathogens in the suspending fluid caused significantly fewer (60-86%) pathogens to adhere, albeit within one hour, supports the theory that if lactobacilli were present or artificially installed on the urethra when a catheter was inserted, the risk of infection should decline. Bacterial competition on the urethra is a dynamic continuous event *in vivo*. The fact that the lactobacilli will remain viable on the urethra, means that they could continue their interference of uropathogenic adhesion over time. This has an advantage over the use of substances such as silver, heparin and antibiotics coated onto catheters, where the antibacterial effect has a time span dependent upon the leaching rate. Further studies are required to test this theory over a longer incubation time and in a clinical setting.

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Discussion with Reviewers

A. Hesse: Do you consider delaying the anti-microbial therapy until clear symptoms appear to be ethically defensible?

Authors: In the case of insertion of an indwelling urinary catheter, we would recommend prophylactic use of an agent such as ciprofloxacin, which, from *in vitro* data, has the capacity to prevent adhesion and kill "young biofilms". We have been most unimpressed with the prophylactic use of co-trimoxazole in patients using intermittent catheterization, thus we would assume it to be equally ineffective for indwelling devices. The duration of prophylaxis then becomes an issue, as long term therapy has side effects and drug resistance problems. The physician must balance risk for the patient with cost, long term care and other factors. It could be asked, what are we trying to prevent when we prescribe these antibiotics? Is it merely catheter colonization, mucosal colonization, prevention of bacteriuria, or

symptomatic infection? New evidence is coming to hand (Reid *et al.*, 1992b; 1993c) which suggests that once a biofilm has formed in the bladder, antibiotic use is only, at best, negating symptoms in 50% of patients and having little effect on the biofilms.

A. Hesse: In your opinion, what form might alternative treatments, which take into consideration the "natural flora", take?
Authors: At present, we would foresee three possible routes for probiotics: (i) coating the actual device with lactobacilli, (ii) coating the external urethra with lactobacilli, (iii) using substances to preferentially stimulate the growth and colonization by normal flora over pathogens. However, for some reason companies have, to date, appeared reluctant to approach these types of alternative treatment methodologies, so it is not clear when and if they will become available for general use.

H.J. Busscher: Is there anything known about how antibiotics influence the surface properties and adhesive ability of the indigenous flora?
Authors: Some data exists which shows that certain antibiotics can damage or depress surface adhesins in uropathogens, thereby inhibiting adhesion. Less is known for indigenous flora, although we have shown that hydrophilic lactobacilli appear less susceptible to vancomycin than hydrophobic organisms, presumably because of some undefined surface properties (Tomeczek *et al.*, 1992).

H.J. Busscher: Could the effects of lactobacillus adhesion as described on the adhesion of uropathogens be due to biosurfactant production of the strains? Such mechanisms have already been described for dairy streptococci and oral streptococci! If yes, attempts would be worthwhile to isolate these biosurfactants.
Authors: The answer in theory is "yes" and "yes". As *L. fermentum* B-54 produces a substance inhibitory to enterococci, it is possible that this is expressed under precoating experimentation, and that this explained why it was the only strain which significantly reduced enterococcal adhesion and survival. This type of question would prove interesting to investigate further.

Reviewer III: Is an 80% reduction in bacterial adherence in the first hour clinically relevant?

Authors: This is an important point, and simply put, the present study was not designed and should not be seen to answer such a specific clinical question. Clearly, we must be careful when extrapolating one hour *in vitro* data in a suspending buffer. Without animal or human tests, or experiments carried out for longer periods in urine, for example, it could always be argued that the results are not important. However, the aim was to reduce initial uropathogen adhesion (not biofilm formation) and this was achieved very effectively. As stated in the discussion, the fact that the lactobacilli are viable, and have been found to effectively compete, long term, with uropathogens on the vaginal mucosa, significantly lowering the incidence of UTI over 12 months (paper submitted), strongly suggests a practical potential for the latest findings. As the reviewer rightly points out, it is the interference on the tissues which will have the most significance. By interfering with the ability of uropathogens to first seed the bladder (either by challenging at the vagina or catheter site), it should be possible to make a clinical impact.

T.A. Fassel: What could account for the difference observed between the *E. coli* and *Enterococcus faecalis* strains in the preferential adhesion experiments? Specifically, do the authors have any thoughts as to why the proportion of adherent enterococci increased in the presence of certain lactobacilli strains but not others?
Authors: Although the water contact angles of these two pathogens are not too dissimilar, the surface characteristics are very different. For example, the *E. coli* strain has protruding fimbriae and the enterococci do not. The enterococci were also more adherent to the catheters than the *E. coli*, possibly explaining why proportionately more enterococci were found, especially in the co-incubation experiments. As stated in the text, co-aggregation between lactobacilli and enterococci is also a possibility.

T.A. Fassel: Do the authors care to speculate on the "factors" produced by *S. epidermidis* and *E. coli* that may alter the suspending fluid and increase lactobacilli adhesion?
Authors: This is too difficult a question to answer with any conviction. One could speculate that substances which aid hydrophobic-hydrophobic or hydrophilic-hydrophilic interactions could be involved, and these could comprise adhesins, extracellular compounds, capsules etc.