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Is it possible to prevent bacterial adhesion onto ureteric stents?

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Abstract The aim of this study was to determine whether the use of bactericidal coatings or immersion in antibiotic solution reduces or prevents bacterial adhesion onto ureteric stents. Precut segments of full silicone, silver-coated and hydrogel-coated ureteric stents were incubated with two uropathogenic bacterial strains with and without previous immersion in antibiotic solution. Tobramycin, ceftriaxone and ciprofloxacin solutions were used, as these antibiotics are commonly administered for the prophylaxis and treatment of urinary tract infection (UTI). Microbiological analysis showed that immersion of ureteric stents in ceftriaxone and ciprofloxacin yielded a significant reduction of bacterial adhesion, whereas immersion in tobramycin did not. The surface material of the stents had no direct influence on bacterial adhesion. In this experimental study, neither the silver nor the hydrogel coat reduced bacterial adhesion onto ureteric stents whereas immersion in a suitable antibiotic solution significantly reduced and even prevented this phenomenon, probably due to the adhesion of the antibiotic onto the stent surface. Prevention of bacterial adhesion onto ureteric stents is essential to reduce the risk of UTI in connection with these devices.

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Introduction

The ability of uropathogens to adhere to the uroepithelium or to the surface of urinary tract prosthetic devices is a major factor in the pathogenesis of urinary tract infection (UTI). In a few hours, adherent bacteria can aggregate, multiply and form biofilm matrices which, once surrounded by a dense glycocalyx, may become resistant to antimicrobial agents and constitute a reservoir of viable microorganisms [1, 3, 12].

Clinical and experimental studies have pointed out that ureteric stents are readily colonised by bacteria in vivo despite antibiotic prophylaxis [2, 5, 8, 13]. Sporadic bacterial adhesion seems to be of little clinical significance whereas formation of biofilms may lead to UTI [2]. The incidence of UTI in patients with indwelling ureteric stents ranges from 8 to 30% [5, 8]. This complication causes morbidity and may even be fatal in patients needing long-term ureteric stenting for malignant disease [8]. Prevention of bacterial adhesion and biofilm formation onto indwelling ureteric stents is therefore important.

A significant reduction of bacterial adhesion onto urethral catheters has been achieved with hydrophilic coating [14], silver or multilayer silver-copper coating [10, 11], salicylic acid impregnation [4], and even electrification [15]. To our knowledge, no attention has been paid to the possibility of preventing or reducing bacterial adhesion onto urinary tract prosthetic devices by dipping them in antibiotic solution before insertion.

The present study was designed to evaluate the occurrence of bacterial adhesion onto ureteric stents with various coatings, and to determine whether it is possible to reduce or prevent bacterial adhesion onto these devices by dipping them in antibiotic solution.

Materials and methods

The adherence of two common uropathogenic bacterial strains, *Escherichia coli* (IH 13047) and *Enterococcus faecalis* (IH 50167), onto three different 5F double-J ureteric stents was studied in vitro. Full silicone (Bard Ureteral Stent, Bard Ltd., Crawley, UK), silver-impregnated polyurethane (Rüsch, Kernen, Germany), and hydrogel-coated C-Flex, (Percuflex Plus, Microvasive, Watertown, USA) were used. The two bacterial species were chosen because of their frequent isolation from biofilms on ureteric stents in vivo [8]. The strains had previously been isolated from the urine of two patients with UTI and had been stored in skimmed milk at −70°C. They were known to adhere onto the surface of biomaterials significantly more than some other bacterial strains previously tested by us [2]. In addition, the strain IH 13047 was known to have virulence-associated characteristics representative of those of most uropathogenic *E. coli* [16].

The bacterial strains were grown overnight at 37°C under gentle shaking in brain heart infusion (BHI) broth. The following day, they were first diluted 1 to 10 and then grown under similar conditions for 2 h. During these 2 h the ureteric stents were cut into 1-cm segments. In sterile conditions, six segments (three for each bacterial strain) of the test items were dropped for 1 min in one antibiotic solution and then placed in sterile tubes containing 5 ml BHI broth, taking care to remove extra drops of the antibiotic solution. Three different antibiotic solutions were used: tobramycin, 80 mg diluted in 100 ml saline; ceftriaxone, 1 g diluted in 100 ml saline; ciprofloxacin, 200 mg in 100 ml. These antibiotics and relative concentrations were chosen because they are those commonly used for the prophylaxis and treatment of UTI. Six other segments (three for each bacterial strain) of the test items were dropped in saline (control solution) and then placed in sterile tubes containing 5 ml BHI broth.

Each tube was inoculated with 50 µl bacterial suspension and incubated for 3 h at 37°C under gentle shaking. Meanwhile, the bacterial suspensions were plated in duplicate onto Luria agar plates after a tenfold serial dilution to calculate the inoculum dose.

After incubation, each catheter segment was washed 5 times by flushing saline, transferred into a sterile Eppendorf tube containing 1 ml saline, and sonicated in a water sonicator for 30 s. After a tenfold bacterial dilution series plated onto Luria agar plates, the number of viable bacteria in saline after sonication was calculated in colony-forming units (CFU). These values represent the maximum number of bacteria able to adhere to the segment during the assay. The adherence of a bacterial strain to a stent segment was calculated with the following formula:

$$\frac{\text{Number of bacteria in saline after sonication}}{\text{Infection dose}} \times 100 = \text{Bacteria capable of adhering (\% of inoculum)}$$

The minimum inhibitory concentration (MIC) of the bacterial strains to the three antibiotics used was tested in vitro by Etest (Ab Biodisk, Solna, Sweden) [18]. The *E. faecalis* strain was sensitive only to ciprofloxacin (MIC 0.75 µg/ml) and resistant to tobramycin (MIC > 256 µg/ml) and to ceftriaxone (MIC > 256 µg/ml). The *E. coli* strain was sensitive to all three antibiotics, with MICs of 0.75 µg/ml to tobramycin, 0.023 µg/ml to ceftriaxone and 0.008 µg/ml to ciprofloxacin.

Statistical analysis

The ANOVA test and Student's *t*-test were used for analysing bacterial adherence to the stent segments. Significance was set at $P \leq 0.05$.

Results

The inoculum dose ranged from 10×10^7 to 14×10^7 CFU/assay. The number of adherent bacteria ranged from 0 to 26×10^4 CFU/segment. Table 1 shows the percentages of the total bacterial inoculum capable of adhering. There was no statistically significant difference in bacterial adhesion onto the various ureteric stents, suggesting that the surface material did not influence bacterial adhesion. This finding allowed us to compare the effects of the various antibiotic solutions on bacterial adhesion, while ignoring the stent materials (Table 2). Immersion in tobramycin did not reduce the adherence of either of the two bacterial strains. Immersion in ceftriaxone prevented the adherence of the *E. coli* strain but had no effect on the *E. faecalis* strain. Immersion in ciprofloxacin significantly reduced the adherence of the *E. faecalis* strain and prevented the adherence of the *E. coli* strain. Overall, immersion of the test items in ceftriaxone and ciprofloxacin yielded a statistically significant reduction of bacterial adhesion, whereas immersion in tobramycin did not (Table 2).

Discussion

Attempts to develop biomaterials that inhibit bacterial adhesion onto urinary tract prostheses have usually

Table 1 Adherent bacteria on precut 1-cm segments of the test items quantified by bacterial culture (the values are percentages of the total bacterial inoculum capable of adhering). Each experiment was done in triplicate

	No antibiotic			Tobramycin			Ceftriaxone			Ciprofloxacin		
Bacterial strains	Silicone	Silver	Hydrogel	Silicone	Silver	Hydrogel	Silicone	Silver	Hydrogel	Silicone	Silver	Hydrogel
	0.418	0.343	0.203	0.493	0.321	1.861	0.364	0.350	0.928	0.005	0.006	0.003
E. faecalis 50167	0.821	1.321	1.143	0.121	0.714	0.857	0.107	0.278	0.031	0.008	0.008	0.004
	1.357	0.214	0.178	0.136	0.228	1.000	0.650	0.257	0.393	0.002	0.001	0.002
Average	0.865	0.626	0.508	0.250	0.421	1.239	0.374	0.295	0.451	0.005	0.005	0.003
	0.155	0.525	0.220	0.355	0.036	0.180	0	0	0	0	0	0
E.coli 13047	0.545	0.435	0.330	0.173	1.050	0.700	0	0	0	0	0	0
	0.355	0.490	0.420	0.048	0.490	1.085	0	0	0	0	0	0
Average	0.352	0.483	0.323	0.192	0.525	0.658	0	0	0	0	0	0

Table 2 Adherent bacteria on precut 1-cm segments of the test items quantified by bacterial culture and grouped according to the different solutions (the values are percentages of the total bacteria inoculum capable of adhering)

Difference in bacterial adhesion onto the test items after immersion in the various solutions:
Ciprofloxacin solution vs other solutions: $P < 0.001$ (ANOVA test)
Tobramycin solution vs control solution: $P = 0.9$ (NS) (Student's t test)
Ceftriaxone solution vs. control solution: $P < 0.01$ (Student's t test)
Ciprofloxacin solution vs control solution: $P < 0.001$ (Student's t test)

Bacterial strains	No antibiotic	Tobramycin	Ceftriaxone	Ciprofloxacin
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E. faecalis 50167	1.321	0.714	0.278	0.008
	0.214	0.228	0.257	0.001
	0.203	1.861	0.928	0.003
	1.143	0.857	0.031	0.004
	0.178	1.000	0.393	0.002
Average	0.666	0.637	0.373	0.004
	0.155	0.355	0	0
	0.545	0.173	0	0
	0.355	0.048	0	0
	0.525	0.036	0	0
E. coli 13047	0.435	1.050	0	0
	0.490	0.490	0	0
	0.220	0.180	0	0
	0.330	0.700	0	0
	0.420	1.065	0	0
Average	0.386	0.455	0	0

focused on urethral catheters whereas little attention has been paid to ureteric stents.

The hydrogel coat and the silver coat did not reduce bacterial adhesion onto ureteric stents in the present study, whereas immersion of these items in ceftriaxone or ciprofloxacin solution reduced or prevented this phenomenon. The tobramycin solution had no effect on bacterial adhesion. This was expected for the *E. faecalis* strain, as the strain was resistant to tobramycin due to the natural resistance of enterococci to aminoglycosides, but unexpected for the *E. coli* strain, as the strain was highly sensitive to tobramycin, and the concentration of tobramycin in the solution was more than 1000-fold the MIC of this *E. coli* strain. This finding suggests that tobramycin was not able to adhere onto any of the stents. The ceftriaxone solution inhibited the adherence of the *E. coli* strain, suggesting that ceftriaxone was able to adhere onto the stents. There was no effect on the *E. faecalis* strain, most likely due to the natural resistance of enterococci to cefalosporins. The ciprofloxacin solution performed best, as it inhibited the adherence of both strains. This was probably due to the fact that both strains were sensitive to ciprofloxacin and suggests that ciprofloxacin was able to adhere onto the stents. Our results suggest that the inhibiting activity of the stents dipped in antibiotic solutions is related to the properties of the antibiotics rather than those of the stent materials.

The adherence of antibacterial substances to medical polymers may, however, be improved. It has been demonstrated that soaking polymers in cold ethanol solution can promote absorption of substances into the polymers [7]. It is then possible to create a slow-release system by adding tridodecylmethylammonium chloride (TDMAC), as the polar part of this molecule binds ionically to the antibacterial substance while the rest binds to the polymer surface [17]. Using this system, up to 60% of the absorbed substance remains bound for 2 weeks [17].

In the case of ureteric stents, a short but strong antibacterial activity is probably preferable to a prolonged but weak one, as it is likely that these devices come into contact with bacteria mainly at the time of their introduction and therefore need bactericidal properties at this stage. This hypothesis is supported by the fact that ureteric stents have been found to be colonised by Gram-positive cocci more frequently than by Gram-negative bacteria [8, 13]. This finding correlates well with previous findings of bacterial adhesion onto urethral catheters [9] and therefore supports the hypothesis of urethral contamination. Moreover, while the incidence of UTI in patients with indwelling urethral catheters rises by 5–10%/day of catheterisation [6], the incidence of UTI in patients with ureteric stents does not correlate with the duration of the stenting [13]. This is probably due to the fact that urethral catheters are external devices on which bacteria may continuously ascend to the bladder, whereas ureteric stents are internal devices unlikely to come into contact with bacteria after their introduction.

In conclusion, prevention of bacterial adhesion onto ureteric stents is essential to reduce the risk of UTI in connection with these devices. The present study indicates that the various coating substances currently available do not provide a significant reduction of bacterial adhesion onto ureteric stents whereas immersion in a suitable antibiotic solution reduces or prevents bacterial adhesion onto ureteric stents. Although of an experimental nature, these findings may be of clinical relevance and provide grounds for further studies in vivo.

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