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# The effect of repeated instillations of antiseptics on catheter-associated urinary tract infections: a study in a physical model of the catheterized bladder

**J. B. King, D. J. Stickler**

School of Pure and Applied Biology, University of Wales, College of Cardiff, Cardiff, UK

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**Summary.** The activity of three antiseptic bladder washout solutions was examined in a physical model of the catheterized bladder. Tests were performed against cultures of four common urinary tract pathogens that had established themselves in the model and colonized the surfaces with biofilm. Double instillations of chlorhexidine (0.02% w/v) at 6-h intervals failed to eliminate *Pseudomonas aeruginosa, Proteus mirabilis,* and *Providencia stuartii* from the bladder model. *Escherichia coli,* however, was susceptible to a second instillation provided that it was performed within 12 h. Supplementing chlorhexidine with EDTA and TRIS potentiated its activity against *E. coli.* Mandelic acid (1.0% w/v) was the most effective of the agents, double instillations eliminating all but *Pr. mirabilis* infections.

**Key words:** Antiseptics – Urethral catheters – Urinary tract infections

Despite many ingenious efforts to improve management prodecures, the urethral catheter remains the most common cause of nosocomial infection [6, 10]. Even with modern drainage systems all patients undergoing long-term indwelling catheterization develop bacteriuria [4, 15, 22]. Invasive infection can follow the bacterial colonization of the catheterized bladder and lead to complications such as pyelonephritis, bladder and renal stones, bacteremia, renal failure, and death [8, 13, 21].

Urinary tract infections in patients undergoing long-term indwelling bladder catherization are difficult to control with antibiotics. While the catheter remains in place, the infecting organisms are only temporarily suppressed by drugs [2] and there is evidence that bacteria growing in biofilm on the catheter surfaces can survive the action of the drugs and reinoculate the urine on completion of the treatment [12]. Washing the bladder with antiseptics has been advocated as an alternative method of control [5], but the rational basis for this procedure is not well established. Using a simple physical model of the catheterized bladder we have previously examined the activity of antiseptic solutions formulated for use in bladder washout procedures [9, 17]. The activity of single instillations of the solutions was examined against organisms that had recently contaminated the bladder urine and also under conditions where organisms had established themselves as stable populations in the model and had colonized the surfaces of the bladder and catheter. The established infections proved to be difficult to eliminate, most bacterial species commonly responsible for catheter-associated infections surviving all attempts to clear them from the bladder. Mandelic acid (1.0 w/v) was the most successful of the solutions; it eliminated *Escherichia coli* from the model, had a marked bactericidal action on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae,* but had only temporary effects on *Proteus mirabilis, Providencia stuartii* and *Streptococcus faecalis.* The purpose of the present study was to examine the activity of instillations repeated at various time intervals on established infections to determine whether they might have a cumulative antibacterial effect.

## Materials and methods

### *The bladder model*

The details of the model of the catheterized bladder have been described previously [18]. In essence, sterile pooled urine is delivered by a peristaltic pump at a rate of 1 ml/min to a fermentation flask (100 ml) which acts as the bladder. The flask is kept at 37°C and the residual volume of urine is maintained at 10 ml by the positioning of the catheter and drainage tube system. Excess urine is drawn off by a vacuum pump to a Buchner flask, which represents the urine drainage bag.

### *Experimental method*

In clinical practice the bladder washout technique involves the introduction of up to 100 ml antiseptic through the catheter into the bladder, where it is retained for 20 min. The model assumes a residual bladder urine of 20 ml and operates at half scale. To

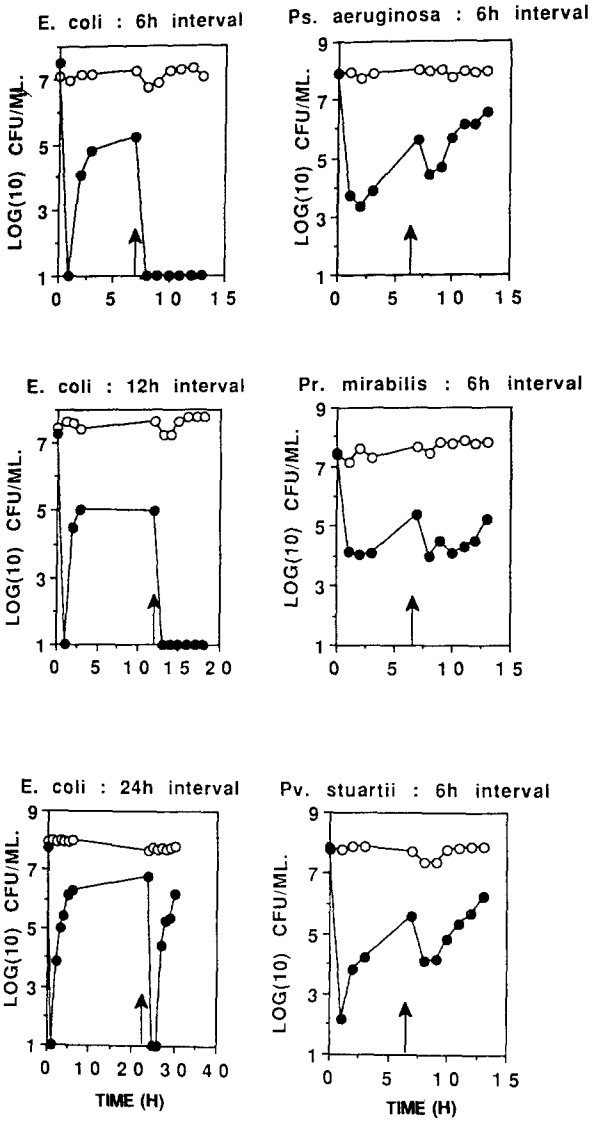


Fig. 1. The activity of double instillations of chlorhexidine (0.02% w/v) on established infections of the four test strains. At time $T=0$ the agent was introduced into the bladder for 20 min. Second instillations were performed at the times indicated by a *vertical arrow*. Viable cell counts were made on the urine at various time intervals (●) and compared with those obtained after control washouts with saline (○). The results shown are the means of two separate experiments

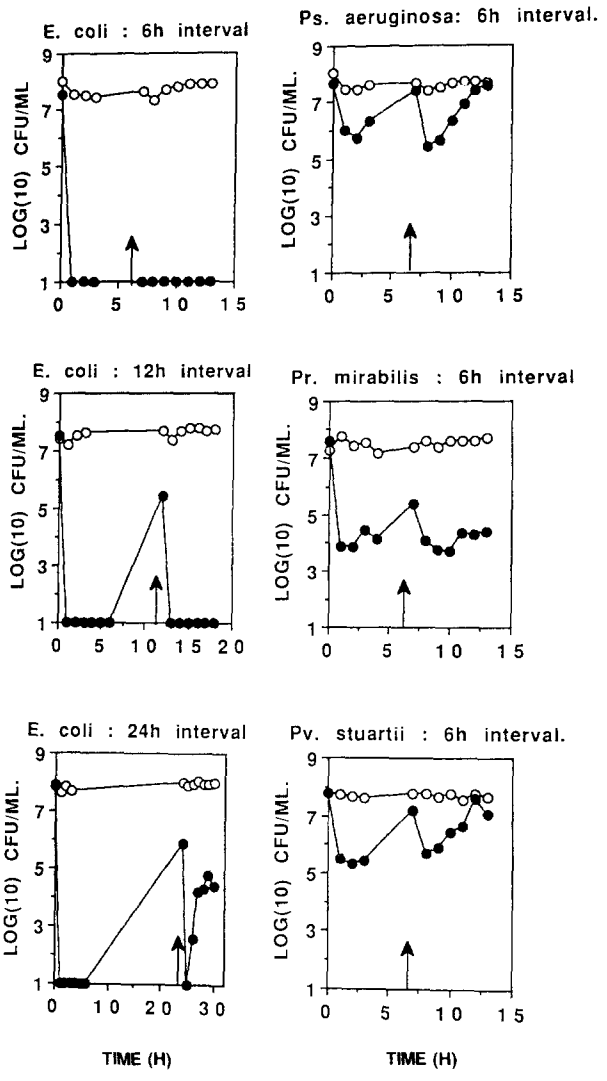


Fig. 2. The activity of double instillations of the chlorhexidine/EDTA/TRIS formulation on established infections of the four test strains. At time $T=0$ the solution was introduced into the bladder for 20 min. Second instillations were performed at the times indicated by a *vertical arrow*. Viable cell counts were made on the urine at various time intervals (●) and compared with those obtained after control washouts with saline (○). The results shown are the means of two separate experiments

simulate established infections, overnight urine cultures (0.1 ml) of the test organisms were inoculated into 10 ml sterile pooled urine in the model. The cultures were incubated for 16 h to allow the walls of the model and the lumen of the catheter to become coated with biofilm and a steady-state population of $10^7$ cfu/ml urine to develop.

To check biofilm formation under these conditions sections of silicone catheter 5 mm in length were incubated in the model for 16 h and then examined by scanning electron microscopy.

The washout procedure was then simulated by instilling 50 ml antiseptic via the catheter into 10 ml contaminated urine. The catheter was clamped and the vacuum pump switched off for 20 min. During this time the supply of urine to the bladder was maintained at 1 ml/min. The clamp was then removed, the vacuum reapplied, and the residual urine returned to 10 ml. Samples of urine were removed from the bladder prior to and immediately after the instillation and at intervals during a subsequent incubation period. Viable cell counts were performed in triplicate on CLED Agar (Oxoid, UK), initial dilutions being made in nutrient broth (Oxoid) containing Tween 80 (3% v/v) to neutralize residual antibacterial activity. Repeat instillations were performed at 6-h to 24-h intervals after which all models were incubated for a further 6 h, viable cell counts being performed at hourly intervals. The test solutions were all supplied by Vifor Medical (Emmenbrucke, Switzerland) in the form of sterile solutions presented in the Urotainer system. They contained (a) mandelic acid 10.0 g/l, sorbitol 40 g/l; (b) chlorhexidine diacetate 0.2 g/l, sorbitol 50.0 g/l; (c) chlorhexidine digluconate 0.2 g/l, EDTA 0.25 g/l, TRIS 6.1 g/l; (d) sodium chloride 9.0 g/l.

With the exception of the reference strain *Escherichia coli* NCTC 10418, the organisms used in this study – *Proteus mirabilis* R368, *Providencia stuartii* R67, *Pseudomonas aeruginosa* R18.11 – were all isolated from the urine of spine-injured patients undergoing bladder catheterization. The strain designations refer to our own reference system.

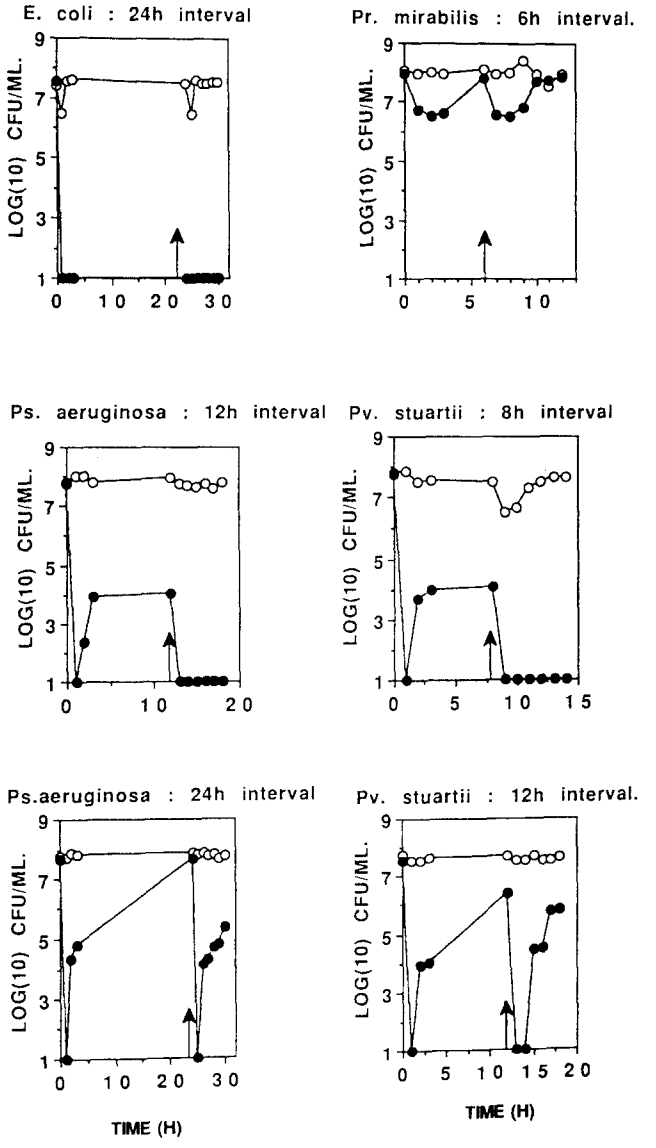


**Fig. 3.** The activity of double instillations of mandelic acid (1.0 w/v) on established infections of the four test strains. At time $T=0$ the agent was introduced into the bladder for 20 min. Second instillations were performed at the times indicated by a *vertical arrow.* Viable cell counts were made on the urine at various time intervals (●) and compared to those obtained after control washouts with saline (○). The results shown are the means of two separate experiments

### *Scanning electron microscopy*

The sections of catheter that had been incubated with the test organisms in the bladder model for 16 h were washed gently in Hanks-Hepes buffer pH 7.4 and placed in a fixative solution consisting of 3% glutaraldehyde in phosphate buffer pH 7.4 for 1 h at 4°C. They were then exposed to osmium tetroxide (1.0%) in phosphate buffer for 1 h and dehydrated in a series of aqueous ethanol solutions (20–100%). Critical point drying was performed in liquid carbon dioxide and finally samples were coated with gold in a sputter coater before examination in a scanning electron microscope (JEOL JS5200).

## Results

The effects of repeated instillations of the three test solutions on the four test strains are presented in Figs. 1–3. Control washouts were performed with saline in each case and the data presented are the mean values from duplicate experiments. It can be seen that single 20-min instillations of chlorhexidine (0.02% w/v) were not effective against established infections of any of the four test species. *Ps. aeruginosa, Pr. mirabilis* and *Pv. stuartii* also resisted double instillations of this agent performed at 6-h intervals. On the other hand *E. coli* was susceptible to a second instillation provided that it was performed within 12 h (Fig. 1). A similar set of results were observed with the chlorhexidine/EDTA/TRIS formulation (Fig. 2). Mandelic acid was the most effective agent, single instillations eliminating *E. coli* and double instillations eliminating all but *Pr. mirabilis* from the bladder (Fig. 3).

Figure 4 presents a series of scanning electron micrographs of biofilms produced on catheter sections during the 16-h incubation period. It can be seen that all four species produce multilayered films on the silicone surfaces.

## Discussion

Previous studies [9] using the physical model of the catheterized bladder have shown that while bladder washout solutions such as chlorhexidine, povidone-iodine, and mandelic acid were effective in clearing recent contamination of the bladder at cell densities of $10^4$ cfu/ml urine, under conditions simulating heavy contamination ($10^7$ cfu/ml) only mandelic acid (1.0 w/v) eliminated the range of bacterial species commonly responsible for catheter-associated urinary tract infection. In those experiments the contaminating bacteria were simply in urine suspension in the bladder. On subsequent incubation of the model the contaminating bacteria established steady-state populations of around $10^7$ cfu/ml and colonized the surfaces of the model wall and the silicone catheters. In the present study, established infections were simulated by incubating the urine cultures in the model for 16 h prior to challenge with the antiseptics. Under these conditions the bacteria colonized the surfaces as thick biofilms of cells embedded in a matrix of polysaccharide material (Fig. 4), a mode of growth which is well known to confer resistance to antibacterial agents [1, 18].

The results presented in Fig. 1 confirm that single instillations of chlorhexidine have little effect on established *E. coli* infections. There was a cumulative effect with the repeated instillations provided that the second instillation was made within 6–12 h. Increasing the time interval to 24 h, however, enabled the cultures to recover sufficiently to survive the second instillation. Single daily washouts with this solution are therefore unlikely to eliminate these sorts of infections in patients. A regimen of repeated instillations at intervals of less than 24 h is required if the bladder is to be cleansed of *E. coli.* The chlorhexidine solution was ineffective against *Ps. aeruginosa, Pr. mirabilis,* and *Pv. stuartii* even when the double

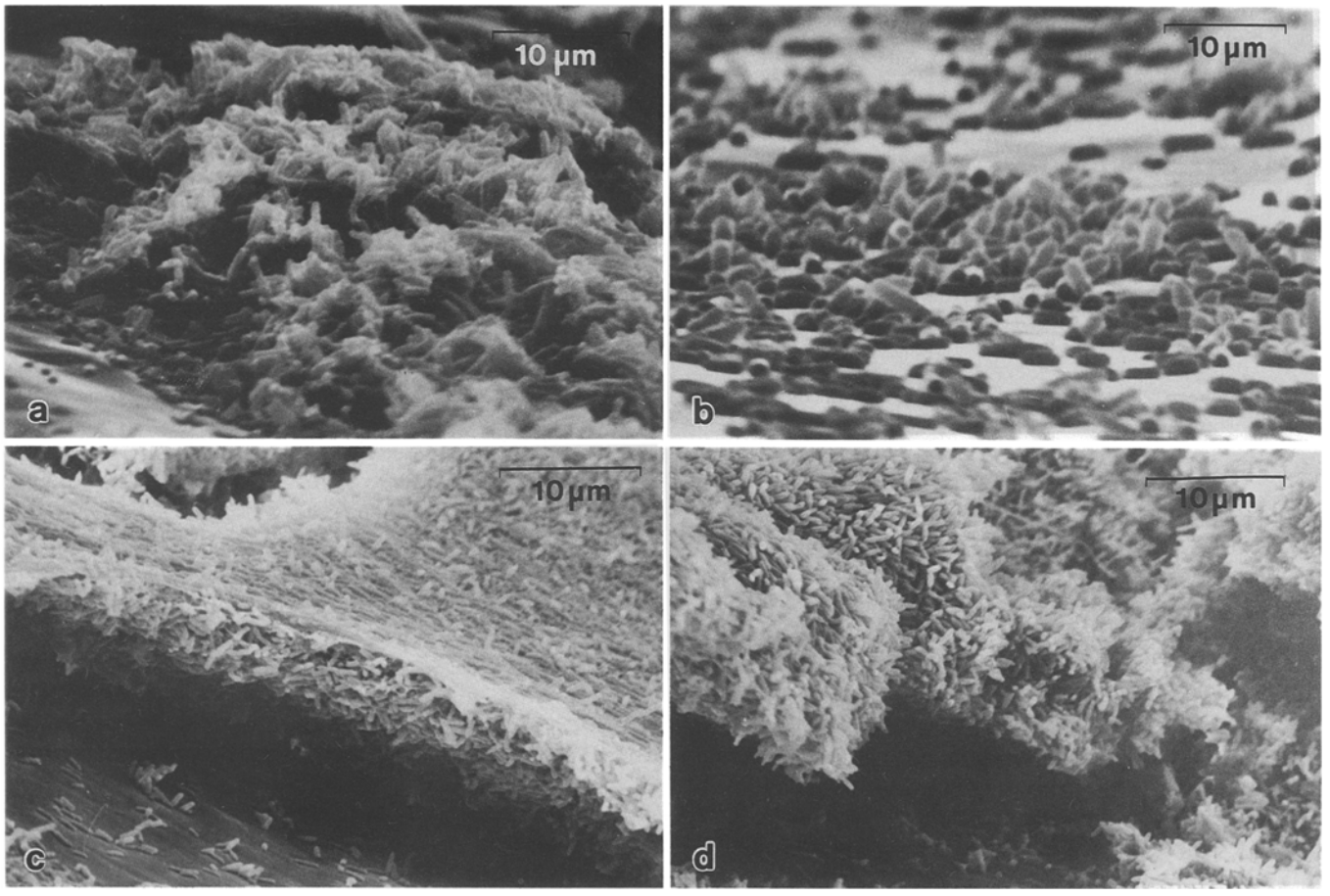


**Fig. 4a–d.** Scanning electron micrographs of sections of catheters after 16 h incubation in the model with the four test strains: **a** *Escherichia coli*; **b** *Proteus mirabilis*; **c** *Pseudomonas aeruginosa*; **d** *Providencia stuartii*

instillations were performed at 6-h intervals. This is not surprising as at 0.02% (w/v) it is not capable of eliminating $10^7$ cfu/ml of these species from urine and has minimal activity against them when they are colonizing silicone surfaces [16, 18, 20]. While chlorhexidine in higher concentrations migh well exhibit a greater bactericidal effect, its irritant effect on the bladder epithelia limits the usable concentration to 0.02% [7].

The addition of EDTA/TRIS to chlorhexidine had little effect on its activity against *Ps. aeruginosa, Pr. mirabilis,* or *Pv. stuartii.* The supplements, however, clearly enhanced the activity against *E. coli* biofilms (Fig. 2). In both experiments *E. coli* cells were not detectable in the urine after a single instillation of the formulation. A second washout at 6 h maintained the sterility of the model. It appears, though, that some cells probably present in the biofilm had in fact survived the first instillation, because when the second instillation was delayed until 12 h they reappeared in the urine. The second instillation eliminated them from the urine however. When the second instillation was performed at 24 h the *E. coli* were only temporarily cleared from the urine and reappeared within an hour. This is an interesting result, as it suggests that the cells in the biofilm were more resistant to the second instillation. Perhaps the challenge of the initial antiseptic washout stimulates the biofilm to produce more protective matrix.

The early work of Rosenheim [14] demonstrated that mandelic acid (1% w/v) was rapidly bactericidal against urine cultures of *E. coli.* As can be seen from Fig. 3, a single instillation of mandelic acid was able to eradicate established infections of *E. coli* from the model. The organism was not detected even after a further 24 h of incubation, which suggests that this agent is active against *E. coli* in both the planktonic and biofilm mode of growth. Biofilms of *E. coli* growing on disks of silicone have previously been shown to be sensitive to mandelic acid [19]. These results all suggest that mandelic acid should be effective against bladder infections of this species in a once-daily regimen.

Previous studies have shown that mandelic acid is bactericidal to *Ps. aeruginosa, Pr. mirabilis,* and *Pv. stuartii* growing as biofilms on silicone disks [20]. In the bladder model the established cultures of these species were slow to recover from the effects of mandelic acid, indicating that the viability of the biofilm had been reduced and its ability to reinoculate the bladder urine impaired. The partial reduction in the viability of the established infections by single 20-min instillations of mandelic acid suggests that repeated instillations might have a cumulative action and eliminate these established infections from the bladder. The results presented in Fig. 3

confirm this cumulative effect in the cases of *Ps. aeruginosa* and *Pv. stuartii.* A second instillation of mandelic acid performed at 6 or 8 h eradicated these organisms from the bladder. At 24 h, however, the partially damaged biofilm seemed to recover its integrity and survived the second challenge.

The results presented in Figs. 1–3 confirm our previous findings and those of others [3] that *Pr. mirabilis* is an extremely difficult organism to shift from the catheterized bladder. Even when the instillations are performed at 6-h intervals the effect on the infections is minimal. This is unfortunate as *Pr. mirabilis* causes particular problems in the catherized bladder, its ability to produce urease and generate alkaline urine resulting in the formation of struvite crystals and subsequent obstruction and blockage of the catheter [11]. It is clear from these results and those reported previously [9] that, if bladder washouts with the currently available solutions are to be effective against this species, they have to be used early in the infection before the organisms have established themselves in the bladder and colonized the available surfaces with biofilm.

It is recognized that in vivo elements such as mucin, and cellular and other organic debris are likely to reduce the activity of these antiseptics. Formulations lacking activity in the clean in vitro test system are thus not likely to be useful in clinical practice. In view of the results presented in Fig. 3 and the previous results showing the activity of mandelic acid against both planktonic and biofilm cells [9, 16, 19] it would be worthwhile investigating the efficacy of mandelic acid bladder washouts in patients.

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J. B. King
School of Pure and Applied Biology
University of Wales
College of Cardiff
Cardiff CF1 3TL
UK