Effect of triclosan on the formation of crystalline biofilms by mixed communities of urinary tract pathogens on urinary catheters

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The crystalline bacterial biofilms that encrust Foley catheters compromise the care of many elderly and disabled patients. The aim of this study was to examine whether the biocide triclosan can prevent encrustation by the mixed flora of uropathogens that commonly infect patients undergoing long-term catheterization. Models of the catheterized bladder were inoculated with communities of organisms isolated from patients who were experiencing catheter blockage. The catheter retention balloons were inflated with water or triclosan (3 g triclosan l⁻¹ in 0.1 M sodium carbonate) and urine was supplied to the models for up to 7 days. The effect of triclosan was recorded on the viable cell populations, the pH of the residual urine and the times that catheters took to block. The extent of encrustation of the catheters was visualized by scanning electron microscopy. In models inoculated with communities containing *Proteus mirabilis*, triclosan prevented the rise in urinary pH that drives crystalline biofilm formation and catheter blockage. The biocide had no effect on populations of *Enterococcus faecalis* and *Pseudomonas aeruginosa*, but *Proteus mirabilis*, *Escherichia coli* and *Klebsiella pneumoniae* were eliminated from the residual urine and the catheters drained freely for the 7-day experimental period. In models inoculated with a mixed community containing *Providencia rettgeri*, catheters inflated with triclosan continued to block rapidly. Although *K. pneumoniae* and *Proteus vulgaris* were eliminated from the residual urine, there was no effect on the viability of *Providencia rettgeri*. The results indicate that the triclosan strategy should be limited to the treatment of patients who are infected with *Proteus mirabilis*.

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INTRODUCTION

The care of many elderly and disabled patients undergoing long-term indwelling bladder catheterization to manage urinary incontinence or retention is frequently complicated by crystalline material blocking their catheters (Kohler-Ockmore & Feneley, 1996). The problem results from infection by urease-producing bacteria, particularly *Proteus mirabilis* (Mobley & Warren, 1987; Kunin, 1989). These organisms colonize the catheters, forming extensive biofilm communities. Their urease activity generates ammonia from urea, elevating the pH of the urine and biofilm. Crystals of calcium and magnesium phosphates then form in the alkaline urine and the biofilm. The continued development of the crystalline biofilms blocks the catheter lumen. In some patients, urine then leaks around the outside of the catheter and patients become incontinent. In others, urine retention causes painful distension of the bladder, and reflux of infected urine through the ureters can precipitate serious symptomatic episodes such as pyelonephritis, septicaemia and endotoxic shock (Kunin, 1997; Morris *et al.*, 1999). All types of Foley catheter are susceptible to this problem and currently there are no

effective procedures available to clinical staff for its control (Morris *et al.*, 1999).

Proteus mirabilis is very sensitive to the biocide triclosan with MICs of 0.2 mg l⁻¹ (Jones *et al.*, 2005). A strategy to control catheter encrustation has been developed in which triclosan can be delivered directly into the residual urine in the catheterized bladder (Stickler *et al.*, 2003). Experiments in laboratory models of the catheterized bladder demonstrated that when the retention balloons of catheters are inflated with solutions of triclosan rather than water, the biocide can diffuse through the balloon membranes into the urine. The bacterial population in the urine is then reduced, the pH of the urine remains acid and the crystalline biofilm does not form on the catheter. This experimental work was performed with pure cultures of *Proteus mirabilis* (Stickler *et al.*, 2003; Jones *et al.*, 2005, 2006). While these single-species biofilms are found on catheters, in long-term catheterized patients, the urine and catheter biofilms are more commonly contaminated by polymicrobial communities frequently containing *Proteus mirabilis* along with other uropathogens (Clayton *et al.*, 1982; Warren *et al.*, 1982; Ohkawa *et al.*, 1990; Ganderton

et al., 1992; Kohler-Ockmore & Feneley, 1996). The aim of the work reported in this paper was to examine the effect of the triclosan strategy on mixed communities of urinary organisms isolated from patients suffering from recurrent catheter encrustation.

METHODS

Media and bacterial strains. The media used in this study were purchased from Oxoid. The bacteria used in the experimental work were clinical isolates from the urine of four patients in community care undergoing long-term catheterization who were experiencing recurrent catheter blockage. They were recovered from clinical samples by culture onto CLED, Chromogenic UTI and tryptone soya agars. After 24 h incubation at 37 °C, the resulting colonies were identified by Gram-staining and the use of the appropriate BBL Crystal identification kits (Becton Dickinson). All isolates were kept as stock cultures in a 5 % (v/v) glycerol solution and stored at -80 °C in Nalgene cryogenic vials. Prior to use in experiments, they were subcultured onto CLED agar. Three species were recovered from each patient as described in Table 1.

MIC determinations. Stock solutions of triclosan (CIBA Speciality Chemicals) were prepared in DMSO (Fisher Scientific) and added to molten Iso-Sensitest Agar (ISA) to produce plates containing a range of triclosan concentrations. Plates containing ISA alone and ISA containing the maximum DMSO concentration (1 %, v/v) were used as controls. Aliquots (10 µl) of a 1:100 dilution of 4 h tryptone soya broth cultures of test organisms (10^4 c.f.u.) were dropped onto the agar plates. MIC was determined as the lowest concentration of triclosan that inhibited bacterial growth after overnight incubation at 37 °C.

Bladder model and experimental protocol. The model of the catheterized bladder has been described previously (Stickler *et al.*,

1999). In essence, it consists of a glass chamber maintained at 37 °C by a water jacket. Each model was sterilized by autoclaving and then a size 14 Ch all-silicone catheter (Bard) was inserted into the chamber via an outlet in its base. Catheters were inflated with 10 ml water, 0.1 M sodium carbonate solution or 3 g triclosan l⁻¹ in a 0.1 M sodium carbonate solution and then attached to drainage tubes and bags. Sets of models were assembled and artificial urine at pH 6.1 described previously by Stickler *et al.* (1999) was pumped into the bladder chamber until it submerged the retention balloons of the catheters (20 ml). The urine supply was then halted and models were inoculated with 1 ml 4 h artificial urine cultures (approx. 10^8 c.f.u.) of each of the three organisms isolated from a particular patient. The models were left for 1 h to enable the organisms to become established in the residual urine. Fresh artificial urine was then pumped into the chamber at 0.5 ml min⁻¹ and left to run for 7 days or until the catheters became blocked. Time to catheter blockage was recorded. Urine samples were removed from the models at various times to measure pH and determine the viable bacterial cell counts on Chromogenic UTI agar.

Low-vacuum scanning electron microscopy. Catheters removed from the models were examined under low vacuum using the 5200 Scanning Electron Microscope (JEOL) to assess the degree of encrustation. Sections 1 cm long were cut from each catheter, placed onto carbon discs on aluminium stubs and viewed without fixation.

Statistical analysis. One-way ANOVA was used at the 95 % significance level to test differences between the means of datasets. Minitab release 13 was used to perform the calculations and to test the normality of distribution of residuals and homogeneity of variances of the data. If any assumptions of ANOVA were violated, the Kruskal-Wallis test was performed at the 95 % confidence interval. Where appropriate the standard error of the mean is indicated.

Table 1. MIC values of triclosan against micro-organisms isolated from the urine of four catheterized patients

This experiment was replicated three times. The ability of each isolate to produce the urease enzyme is also shown.

Isolate	Triclosan MIC (mg l ⁻¹)	Urease
Urine community 1		
<i>Ent. faecalis</i> P9	4	No
<i>E. coli</i> P9	0.2	No
<i>P. mirabilis</i> P9	0.1	Yes
Urine community 2		
<i>Ent. faecalis</i> P11	4	No
<i>K. pneumoniae</i> P11	0.2	Yes
<i>P. mirabilis</i> P11	0.1	Yes
Urine community 3		
<i>Ent. faecalis</i> SM42	4	No
<i>Ps. aeruginosa</i> SM63	>1000	Yes
<i>P. mirabilis</i> SM46	0.1	Yes
Urine community 4		
<i>Prov. rettgeri</i> SDM1	25	Yes
<i>P. vulgaris</i> SDM2	0.1	Yes
<i>K. pneumoniae</i> SDM3	0.2	Yes

Table 2. Effect of inflating retention balloons with triclosan on ability of mixed communities of urinary tract pathogens to encrust and block all-silicone catheters

Models were fitted with all-silicone catheters inflated with water, 0.1 M sodium carbonate solution or 3 g triclosan l⁻¹ in 0.1 M sodium carbonate and inoculated with polymicrobial communities isolated from the urine of four catheterized patients. Models were supplied with urine for up to 7 days. The values are from experiments replicated three times. There was no significant difference in the mean times to blockage for community 4 in the presence and absence of triclosan ($P > 0.05$).

Urine community	Mean time to blockage (h ± SE) after inoculation with natural communities		
	Water control	Sodium carbonate control	Triclosan test
1	40.5 ± 3.9	38.4 ± 2.4	>168
2	31.9 ± 1.9	34.6 ± 3.2	>168
3	30.9 ± 1.7	36.7 ± 1.4	>168
4	31.7 ± 5.2	31.9 ± 4.7	22.1 ± 0.9

Fig. 1. Effect of triclosan on the pH of the bladder urine in models inoculated with polymicrobial communities isolated from the urine of four catheterized patients. The pH of urine was measured at catheter blockage or at 7 days in those models where the catheter drained freely for the full experimental period. The values are from experiments replicated three times.

RESULTS AND DISCUSSION

Susceptibility of urinary tract pathogens to triclosan

The MICs of triclosan for the micro-organisms that had been recovered from the urine of four catheterized patients are presented in Table 1. The ability of the isolates to produce urease is also indicated. The results show that the isolates of *Proteus mirabilis*, *Proteus vulgaris*, *Escherichia coli* and *Klebsiella pneumoniae* were all highly susceptible to triclosan (MICs 0.1–0.2 mg l⁻¹). The *Enterococcus faecalis* isolates were less susceptible (MIC 4 mg l⁻¹) and the *Pseudomonas aeruginosa* was resistant to the highest concentration of triclosan tested (1000 mg l⁻¹). Overall, these results are

consistent with earlier findings (Bhargava & Leonard, 1996; Jones *et al.*, 2006). We could find no information in the literature on the susceptibility of *Providencia rettgeri* to triclosan but the isolate from community 4 clearly exhibited a degree of resistance (MIC 25 mg l⁻¹). It is interesting that Jones *et al.* (2006) reported that *Providencia stuartii* was sensitive to triclosan (MIC 0.2–0.4 mg l⁻¹).

Effect of triclosan on the ability of the mixed bacterial communities to form crystalline biofilms on catheters

In each experiment, sets of three models were fitted with all-silicone catheters. In the two control models, the catheter retention balloons were inflated with either sterile

Table 3. Effect of triclosan on the viable cell numbers of the uropathogens in urine

The viable cell count (log₁₀ c.f.u. ml⁻¹ ± SE) of bacteria in the residual urine was measured at the start of experiments (0 h) and at catheter blockage or at 7 days in those models where the catheter drained freely for the full experimental period. The values are from experiments replicated three times.

Species	Water control		Sodium carbonate control		Triclosan test	
	Start	Blockage	Start	Blockage	Start	168 h
Community 1						
<i>Ent. faecalis</i> P9	7.24 ± 0.04	8.16 ± 0.17	7.17 ± 0.05	7.89 ± 0.29	6.8 ± 0.02	7.41 ± 0.18
<i>E. coli</i> P9	6.21 ± 0.3	4.97 ± 0.66	6.46 ± 0.37	5.11 ± 0.29	6.2 ± 0.44	<2
<i>P. mirabilis</i> P9	6.9 ± 0.06	7.59 ± 0.17	6.71 ± 0.17	7.22 ± 0.12	6.31 ± 0.14	<2
Community 2						
<i>Ent. faecalis</i> P11	7.07 ± 0.06	8.24 ± 0.03	7.12 ± 0.04	8.01 ± 0.13	6.99 ± 0.04	7.03 ± 0.21
<i>K. pneumoniae</i> P11	7.71 ± 0.05	4.74 ± 0.07	7.79 ± 0.01	5.12 ± 0.17	6.36 ± 0.21	<2
<i>P. mirabilis</i> P11	7.33 ± 0.04	7.48 ± 0.06	7.51 ± 0.1	7.49 ± 0.14	6.91 ± 0.12	<2
Community 3						
<i>Ent. faecalis</i> SM42	6.49 ± 0.24	7.65 ± 0.06	6.45 ± 0.35	7.37 ± 0.20	6.03 ± 0.2	8.11 ± 0.14
<i>Ps. aeruginosa</i> SM63	7.04 ± 0.08	6.93 ± 0.15	6.96 ± 0.1	7.12 ± 0.16	6.8 ± 0.08	7.54 ± 0.14
<i>P. mirabilis</i> SM46	7.12 ± 0.14	7.37 ± 0.19	7.17 ± 0.14	7.21 ± 0.14	6.17 ± 0.29	<2
Community 4						
<i>Prov. rettgeri</i> SDM1	5.9 ± 0.32	6.26 ± 0.22	5.87 ± 0.39	6.36 ± 0.22	5.86 ± 0.19	6.05 ± 0.02‡
<i>P. vulgaris</i> SDM2	7.09 ± 0.04	7.01 ± 0.13	7.09 ± 0.03	6.87 ± 0.3	5.65 ± 0.02	<2‡
<i>K. pneumoniae</i> SDM3	7.63 ± 0.04	4.39 ± 1.87*	7.68 ± 0.05	5.7†	6.48 ± 0.09	<2‡

*Indicates that a viable cell count was only detected in two replicates.

†Indicates that a viable cell count was only detected in one replicate.

‡Indicates that the viable cell counts of bacteria in urine community 4 were measured at catheter blockage.

Fig. 2. Scanning electron micrographs of control all-silicone catheters inflated with water and test catheters inflated with 3 g triclosan l^{-1} in 0.1 M sodium carbonate solution. (a–d) Cross-sections of control catheters taken from models inoculated with communities 1–4. (e–h) The same views of triclosan test catheters.

deionized water or sodium carbonate solution (0.1 M). In the test models, catheters were inflated with the triclosan solution (3 g l^{-1} in 0.1 M sodium carbonate). Each set of models was then inoculated with one of the three-membered bacterial communities and urine was supplied to models for 168 h or until catheters blocked. These experiments were used to determine whether the diffusion of triclosan through catheter retention balloons can prevent the formation of polymicrobial catheter-blocking biofilms and to monitor for shifts in the microbial flora in the bladder urine.

The mean times that catheters took to block are presented in Table 2. The strategy was clearly effective in preventing blockage by communities 1–3. In these cases, catheters loaded with triclosan drained freely for the full experimental period. The control catheters all blocked rapidly, confirming the ability of these communities to produce

crystalline biofilm. The mean pH values of the residual urine in the models after 168 h or at the time of catheter blockage are presented in Fig. 1. In the control models inoculated with communities 1–3, the urinary pH rose from 6.1 to mean values above 8 at blockage. Inflating the balloons with triclosan, however, resulted in the urine remaining acidic for the duration of the experiments. The mean numbers of viable cells recovered from these urines are presented in Table 3. Inflating the catheters with the triclosan solution substantially reduced the numbers of viable *Proteus mirabilis* cells in the urine, no viable cells being detected in the test models for the duration of the 7-day experiments.

The effect of triclosan on the urine cultures in the bladder model reflected the triclosan MIC values. Isolates with an MIC in agar of ≥ 4 mg l^{-1} were not eliminated from the urine of the bladder model. Thus the highly sensitive *E. coli*

isolate (community 1) and *K. pneumoniae* isolate (community 2) were reduced to below detectable levels (Table 3). There were initial reductions in the numbers of the *Enterococcus faecalis* isolates after 24 h in communities 1–3 (data not shown), but the numbers began to recover by the end of the 7-day experiments. There was no significant reduction ($P > 0.05$) in the numbers of the highly resistant *Pseudomonas aeruginosa* isolate (community 3) in the presence of triclosan.

Scanning electron micrographs of the biofilms found on control and triclosan test catheters are presented in Fig. 2. They confirm that catheters removed from control models inoculated with communities containing *Proteus mirabilis* blocked with crystalline biofilm (Fig. 2a–c). When *Proteus mirabilis* was eliminated from the bladder urine in triclosan test models, none of the catheters became colonized with crystalline biofilm (Fig. 2e–g). The urease-producing *Pseudomonas aeruginosa* isolate (community 3) was not capable of producing alkaline urine (Fig. 1) and crystalline biofilm (Fig. 2g). This is consistent with previous findings (Stickler *et al.*, 1998; Jones *et al.*, 2006; Macleod & Stickler, 2007).

An earlier *in vitro* study by Stickler *et al.* (1998) showed that urease-producing isolates of *Proteus vulgaris* and *Providencia rettgeri* could generate alkaline conditions and form crystalline biofilms. It was not surprising, therefore, that a community containing both these species (community 4) was capable of rapidly generating catheter-blocking biofilms (Table 2). When bladder models were inoculated with *Providencia rettgeri*, *Proteus vulgaris* and *K. pneumoniae*, there was no significant difference in the mean time to blockage of catheters in the presence and absence of triclosan ($P > 0.05$). Urinary populations of the triclosan-susceptible isolates *Proteus vulgaris* and *K. pneumoniae* were eliminated below detectable levels in the urine of test models (Table 3). Triclosan had no significant effect ($P > 0.05$) on the viable cell numbers of the *Providencia rettgeri* isolate (Table 3). The pH in both control and test models rapidly rose from 6.1 to >8 (Fig. 1) and scanning electron microscopy confirmed that the catheters were heavily encrusted (Fig. 2d, h).

The study confirms that the strategy of inflating catheter retention balloons with triclosan prevents catheter encrustation by *Proteus mirabilis* and can eliminate several other common pathogens from the urine, including *Proteus vulgaris*, an organism also capable of generating catheter-blocking biofilm. The results also suggest that the MICs of triclosan for strains capable of forming crystalline biofilm will be a good indication of the likely success of the strategy. While it will obviously be necessary to test the susceptibility of other strains of *Providencia rettgeri* to triclosan, the results presented in Tables 2 and 3 indicate that loading catheters with triclosan may not prevent catheter blockage by this organism. It should be emphasized, however, that *Proteus mirabilis* is the species most commonly responsible for catheter encrustation (Mobley & Warren, 1987; Stickler *et al.*, 1993).

The results indicate that if triclosan is used in a general strategy to prevent catheter encrustation in patients undergoing long-term catheterization, it is likely that species less susceptible to the biocide will be selected from the polymicrobial urine communities. It is also possible that resistance to triclosan might develop in previously sensitive species in response to extensive use of the agent (Stickler & Jones, 2008). We suggest that because of these risks triclosan should be reserved to treat patients who are infected with *Proteus mirabilis* and suffering the complication of catheter encrustation. It will also be important in any clinical trial or subsequent clinical use of the strategy to monitor the urinary flora of the catheterized patients being treated for signs of the emergence of less susceptible strains or the selection of intrinsically resistant species.

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