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Crystalline bacterial biofilm formation on urinary catheters by urease-producing urinary tract pathogens: a simple method of control

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The problem of catheter encrustation stems from infection by urease-producing bacteria. These organisms generate ammonia from urea, elevate the pH of urine and cause crystals of calcium and magnesium phosphates to form in the urine and the biofilm that develops on the catheter. In this study, a laboratory model was used to compare the ability of 12 urease-positive species of urinary tract pathogens to encrust and block catheters. *Proteus mirabilis*, *Proteus vulgaris* and *Providencia rettgeri* were able to raise the urinary pH above 8.3 and produce catheter-blocking crystalline biofilms within 40 h. *Morganella morganii* and *Staphylococcus aureus* elevated the pH of urine to 7.4 and 6.9, respectively, and caused some crystal deposition in the biofilms but did not block catheters in the 96 h experimental period. Isolates of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Providencia stuartii* were only capable of raising the pH of urine to a maximum of 6.4 and failed to cause crystal deposition in the biofilm. The most effective way to prevent catheter encrustation was shown to be diluting urine and increasing its citrate concentration. This strategy raises the nucleation pH (pH_n) at which calcium and magnesium phosphates crystallize from urine. Increasing the fluid intake of a healthy volunteer with citrated drinks resulted in urine with a pH_n of >8.0 in which catheter encrustation was inhibited. It is suggested that this dietary strategy will be an effective means of controlling catheter encrustation, whichever bacterial species is causing the problem.

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INTRODUCTION

A common complication in the care of patients undergoing long-term indwelling bladder catheterization is encrustation and blockage of the catheter (Stickler & Zimakoff, 1994). It can result in emergency referrals of patients in acute discomfort with urinary retention or incontinent of urine because of sudden catheter blockage (Kohler-Ockmore & Feneley, 1996). The problem stems from infection by urease-producing bacteria that colonize the catheter forming extensive biofilms. The bacterial urease generates ammonia from urea, elevating the pH of urine and biofilm. As the pH of urine rises, crystals of calcium and magnesium phosphates come out of solution. The pH at which this occurs is known as the nucleation pH (pH_n) (Choong *et al.*, 2001). In patients who develop infections with urease-producing bacteria, the pH of the voided urine (pH_v) can thus rise above the pH_n and crystallization occurs in the urine and the biofilm. The continued development of this crystalline biofilm blocks the flow of urine through the catheter (Morris *et al.*, 1999).

Abbreviations: pH_n, nucleation pH; pH_v, pH of the voided urine.

All available types of indwelling catheters are vulnerable to this problem (Morris *et al.*, 1997) and currently there are no effective procedures available for its control (Kunin, 1997).

There is strong epidemiological and experimental evidence that *Proteus mirabilis* is a major cause of catheter encrustation (Mobley & Warren, 1987; Kunin, 1989; Stickler *et al.*, 1993). A strategy has been developed to prevent crystalline biofilm formation by this organism. It simply involves diluting urine and increasing its citrate concentration. This elevates the pH_n of urine above the pH which *P. mirabilis* normally generates in urine (Stickler & Morgan, 2006). A wide range of organisms will colonize the urine and catheters of patients undergoing long-term catheterization (MacLeod & Stickler, 2007) and several are capable of producing urease in standard bacteriological identification tests. A preliminary study in laboratory models by Stickler *et al.* (1998) found that of these other species, *Proteus vulgaris* and *Providencia rettgeri* were capable of generating alkaline urine and producing crystalline biofilms over 24 h incubation periods. The aims of the present study were to (a) test the ability of these

organisms and an extended range of urease-producing species to block catheters; (b) examine the effect of the citrate strategy on catheter encrustation by crystalline biofilm-forming species; and (c) test whether by increasing fluid intake of a healthy volunteer with citrated drinks it was possible to raise the pH_n of urine above the pH_v generated by urease-positive species, thus producing urine in which catheter encrustation is inhibited.

METHODS

Bacterial strains and culture media. The organisms used in this study were isolated from the biofilms colonizing catheters of patients undergoing long-term bladder catheterization (MacLeod & Stickler, 2007). They had all been screened for urease activity on urea agar slopes. The artificial urine used in the experimental work was based on that devised by Griffith *et al.* (1976). It contained calcium chloride 0.49 g l^{-1}, magnesium chloride hexahydrate 0.65 g l^{-1}, sodium chloride 4.6 g l^{-1}, di-sodium sulphate 2.3 g l^{-1}, tri-sodium citrate dihydrate 0.65 g l^{-1}, di-sodium oxalate 0.02 g l^{-1}, potassium dihydrogen phosphate 2.8 g l^{-1}, potassium chloride 1.6 g l^{-1}, ammonium chloride 1.0 g l^{-1}, urea 25 g l^{-1}, gelatin 5.0 g l^{-1}. The pH of the medium was adjusted to 6.1 and then the medium was sterilized by membrane filtration. Tryptone soya broth (Oxoid) was prepared separately, autoclaved and added to the sterile basal medium to a final concentration of 1.0 g l^{-1}. When citrate was added, the urines were adjusted to this pH 6.1. CLED agar (Oxoid) was used for the enumeration of viable cells in urine.

The bladder model. The bladder model 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 through an outlet at the base. The catheter retention balloons were inflated with 10 ml sterile water and the catheters were connected to drainage bags in the normal way. Sterile artificial urine was pumped into the chambers so that residual volumes collected below the catheter eye-holes before flowing through the drainage tube to the collecting bags.

Experimental protocol. Sets of models were assembled and supplied with artificial urine up to the level of the catheter eye-holes. The urine supply was then switched off and 10 ml of the artificial urine was removed from the bladder chamber and replaced with a 4 h artificial urine culture (10 ml) of the various test organisms. These cultures were left for an hour to establish themselves in the bladder, and then the urine supply was resumed until catheter blockage. The times taken for the catheters to block were recorded. The urinary pH and the numbers of viable cells in the urine voided from the model were measured at intervals up to 7 days or the time of catheter blockage.

Scanning electron microscopy of catheter sections. Catheters were removed from the models and sections (1 cm in length) were cut from the region above the retention balloon. In some cases, these sections were examined directly at low power using the low vacuum facility of a JEOL 5200 scanning electron microscope. Other sections were perfusion-fixed in 2.5 % glutaraldehyde in 0.1 M Sörensen buffer (pH 8) (4 h at room temperature or overnight at 4 °C). Subsequently catheter sections were washed in the buffer for 15 min before post-fixing using a 1 : 1 solution of 0.05 M buffer, 1 % osmium tetroxide for 1 h. A further 15 min wash in distilled water was carried out before samples were dehydrated in an ascending ethanol series. Sections were then dried by sublimation dehydration using hexamethyldisilizane (HMDS), i.e. 70 %, 90 %, 100 %, 100 % ethanol, 100 % ethanol : HMDS (1 : 1). Finally, they were washed twice in 100 % HMDS (15 min each step) and left to dry in air. In some cases, sections were dried using a critical point drier (Blazers CPD 030, Bal-Tec Ag). The samples were gold sputter-coated (Edwards S150P sputter-coater) and visualized using a Philips XL-20 scanning electron microscope, accelerating voltage 20–25 kv.

Urease assay. Test organisms were grown for 4 h at 37 °C with aeration in artificial urine. The cells were harvested by centrifugation and then resuspended in 0.1 M sodium phosphate buffer (pH 7.3) containing 10 mM EDTA. The assay based on the modified Bertholt reaction (Creno *et al.*, 1970) was used to determine the urease activity of the whole-cell suspension. Cell suspensions (200 µl) were added to a reaction mixture containing 50 mM urea and 100 mM sodium phosphate buffer (pH 7.3) and incubated at 37 °C for 10 min. The reaction was terminated by the addition of 2 ml of a solution of phenol (0.5 %) and sodium nitroprusside (0.0025 %). Subsequently 2 ml of a solution of sodium hydroxide and sodium hypochlorite (0.21 %) was added and colour development was allowed to proceed for 6 min at 56 °C. The OD_{600} was measured against a reagent blank and the amount of ammonia and therefore the amount of urea hydrolysed were calculated from standard curves prepared using solutions of ammonium chloride. Protein levels in the cell suspensions were determined using commercial protein determination kits (Sigma). The urease activity was then expressed as µmol urea hydrolysed min^{-1} (mg protein)$^{-1}$.

Determination of the pH_n of urine. Evaluation of pH_n was based on the method described by Choong *et al.* (1999). Urine was maintained at 37 °C, and samples were alkalinized in increments of 0.20 pH units with 1 M sodium hydroxide solution up to a pH of 10. At each increment, the OD_{600} was measured against urine at pH 5.0 as a blank with a Unicam Helios γ spectrophotometer. The pH_n was determined from the resulting plot of pH versus optical density. The pH_n was defined by an abrupt change in the slope of the graph showing an increase in turbidity. Plotting pH versus optical density produces two straight-line segments which intersect at the pH_n. Regression lines were calculated by least-squares analysis for these two portions of the graph and used to determine the pH at their intersection.

Determination of the rate of calcium and magnesium deposition on catheters. At the end of each experimental period, catheters were removed from the models, the inflation balloons were dissected away from the catheters and the whole length of the catheter was cut into 1 cm sections. These sections were placed into 100 ml 4 % (v/v) nitric acid in double deionized water and sonicated at 35 kHz for 5 min in the Transsonic water bath to aid biofilm removal and disruption. Samples were stood for at least 48 h to allow the crystals to dissolve. The calcium and magnesium content of the resulting solutions was then assayed using flame atomic absorption spectroscopy in a SpectrAA-100 spectrophotometer (Varian) calibrated with Spectrosol standards obtained from VWR International. Samples were aspirated into the flame (air–acetylene for magnesium and nitrous oxide–acetylene for calcium). Calcium was measured at 422.7 nm and magnesium at 285.2 nm. The rate of catheter encrustation was then expressed as µg Ca and Mg deposited per catheter h^{-1}.

Statistical analysis. One-way ANOVA carried out at a 95 % confidence interval was the statistical test of choice for all the experiments. This was carried out using Minitab release 13 software. Where appropriate, the standard error (SE) of the mean is indicated.

RESULTS AND DISCUSSION

The ability of urease-positive isolates of 11 species of urinary tract pathogens to block catheters in the bladder

model was examined over 96 h test periods in which the basic artificial urine was supplied to the models at 0.5 ml min^{-1}. Urease-negative isolates of *Escherichia coli* and *Staphylococcus aureus* were also included in the study for comparison. The results from triplicate experiments are presented in Table 1. In the case of the control urease-negative organisms, the urinary pH remained acid at around 6.1. All the urease-positive species raised the pH of urine significantly ($P < 0.05$) in the model over the incubation period. The isolates fell into three distinct groups. *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Providencia stuartii* generated urinary pHs of 6.2–6.4. *Staph. aureus* and *Morganella morganii* produced urinary pHs of 6.9–7.4. *P. mirabilis*, *P. vulgaris* and *Pv. rettgeri* generated considerably more alkaline urine (pH 8.3–8.4) and caused the rapid blockage of the catheters. None of the other isolates produced blockage over the 96 h. Photographs of catheters taken from the models at blockage or 96 h (Fig. 1) show the extent of encrustation around the catheter eye-holes and central channels.

The pH_n of the urine supplied to the models was 6.7, explaining why there were no signs of crystal deposition on catheters colonized by the urease-negative organisms and by those urease-positive species only capable of elevating the urinary pH to 6.2–6.4 (Fig. 2). As the *Staph. aureus* and *M. morganii* isolates elevated the pH above the pH_n, a degree of crystallization was found in their biofilms at 96 h (Fig. 3c, d). Encrustation was obviously less extensive, however, than in the cases of those species capable of producing urinary pH values above 8.3 and blocking the catheters within 36 h (Fig. 3a, b).

Table 1. Times catheters took to block and urinary pH values in models inoculated with a range of urease-producing isolates

Organism	Time (h) to catheter blockage (± SE)*	pH of urine at blockage or 96 h (± SE)
Proteus mirabilis RB6	19.8 (2.9)	8.34 (0.19)
Proteus vulgaris SDM2	36.4 (3.9)	8.42 (0.21)
Providencia rettgeri SDM1	32.2 (6.2)	8.36 (0.19)
Morganella morganii RB15	–	7.39 (0.08)
Staphylococcus aureus P10 6/9	–	6.89 (0.06)
Providencia stuartii RB14	–	6.44 (0.06)
Pseudomonas aeruginosa RB16	–	6.32 (0.05)
Klebsiella pneumoniae SDM17	–	6.31 (0.05)
Serratia marcescens†	–	6.18 (0.01)
Klebsiella oxytoca†	–	6.24 (0.01)
Enterobacter cloacae†	–	6.20 (0.02)
Staphylococcus aureus W112 urease −ve	–	6.09 (0.01)
Escherichia coli RB13 urease −ve	–	6.15 (0.02)
Uninoculated control	–	6.11 (0.01)

*–, Indicates that the catheters did not block in the 96 h test period.
†In these cases, the mean values were calculated from those obtained from single experiments with two to three different isolates.

Although *K. pneumoniae* did not generate crystalline biofilm, it did produce mucoid plugs in parts of the catheter lumen (Fig. 1). Similar plugs were produced by *Ps. aeruginosa* (not shown). While these did not completely block the catheters, they did restrict the flow of urine. Poorly draining catheters in patients are occasionally obstructed by mucus. Examination of this material might reveal it to be mucoid bacterial biofilm.

The amounts of calcium and magnesium deposited on catheters removed from the models at blockage or 96 h were determined by flame atomic absorption spectroscopy. The overall rates of encrustation were calculated and the results are presented in Table 2. The three species that blocked catheters clearly produced the most rapid encrustation. Of the other organisms, only *M. morganii* produced appreciable rates above baseline. The urease activity of 4 h urine cultures of the test organisms is also presented in Table 2. These results show that while species such as *K. pneumoniae*, *Ps. aeruginosa*, *Pv. stuartii* and *Staph. aureus* can produce urease on urea agar in standard bacteriological identification tests, the activity that they produce in urine is substantially less than that produced by *P. mirabilis*, *P. vulgaris*, *Pv. rettgeri* and *M. morganii*.

Antimicrobial catheters (the silver/hydrogel-coated latex Bard IC catheter and the nitrofurazone-impregnated all-silicone catheter, Rochester Medical NF catheter) have been introduced recently into clinical practice. It has been shown that *P. mirabilis* is capable of encrusting and blocking these antimicrobial catheters *in vitro* and *in vivo* (Stickler & Morgan, 2008; Morgan *et al.*, 2009). Experiments were performed in the bladder models to test the abilities of the other species that were capable of forming crystalline biofilm in urine to encrust and block these catheters. All-silicone catheters were used as controls. The models were run until catheters blocked or for a total of 168 h. The results presented in Table 3 show the rapid blockage of the silicone and the silver and nitrofurazone catheters by *P. mirabilis*, *P. vulgaris* and *Pv. rettgeri*. *Staph. aureus* failed to block any of the catheters during the incubation period. The results show that given the longer incubation period of 168 h, *M. morganii* was capable of blocking all-silicone and NF catheters. The silver catheter, however, did not block. It is clear from the images presented in Fig. 4, however, that while there was little sign of crystalline biofilm formed by *Staph. aureus* on the antimicrobial catheters, the other four species all produced extensive encrustation on both these catheters, and *M. morganii* must have been very close to blocking the silver catheters at 168 h.

Previous studies have shown that *P. mirabilis* is very sensitive to the biocide triclosan (Stickler, 2002). A strategy to control crystalline biofilm formation by this organism was thus developed which involved inflating the catheter retention balloon with triclosan solution rather than water.

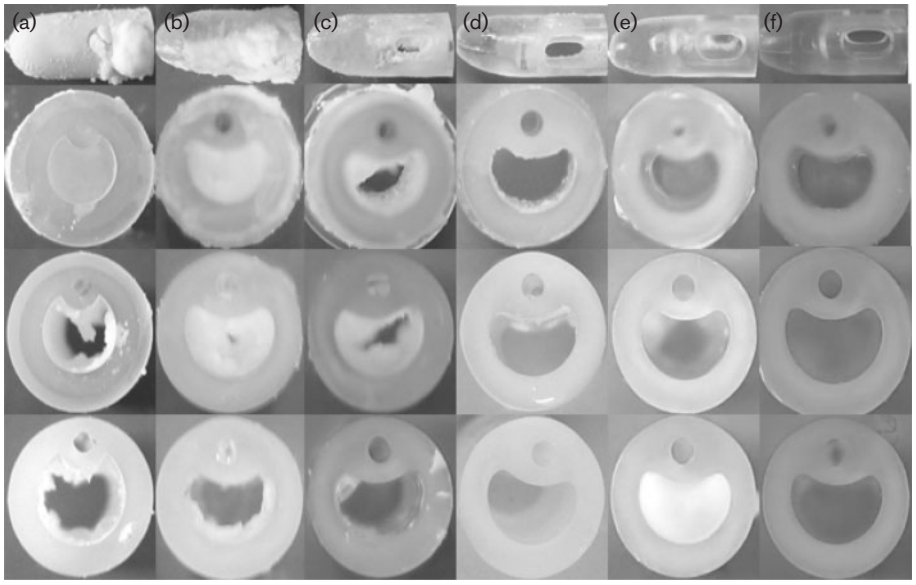

Fig. 1. Each column shows the eye-holes and a series of cross-sections taken along the length of catheters removed from models that had been inoculated with (a) *P. vulgaris* SDM2, (b) *Pv. rettgeri* SDM1, (c) *M. morganii* RB15, (d) *Staph. aureus* P10 6/9, (e) *K. pneumoniae* SDM17 and (f) *E. coli* RB13. The catheters in (a) and (b) were removed from models at blockage; those in (c)–(f) were removed after 4 days incubation. *E. coli* was included as a urease-negative organism; all other organisms shown were urease producers.

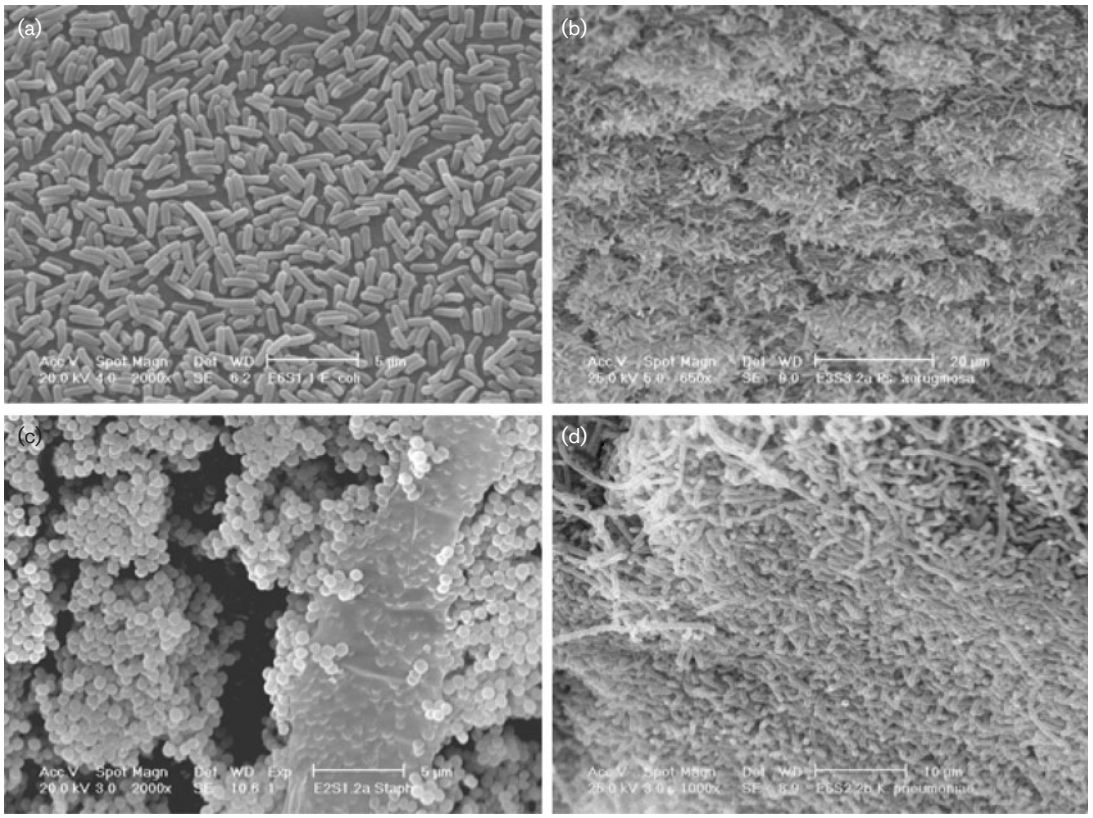

Fig. 2. Scanning electron micrographs of biofilms growing on catheters removed from models after 4 days incubation. (a) Urease-negative *E. coli* RB13; (b) urease-positive *Ps. aeruginosa* RB16; (c) urease-negative *Staph. aureus* (W112); and (d) urease-positive *K. pneumoniae* SDM17.

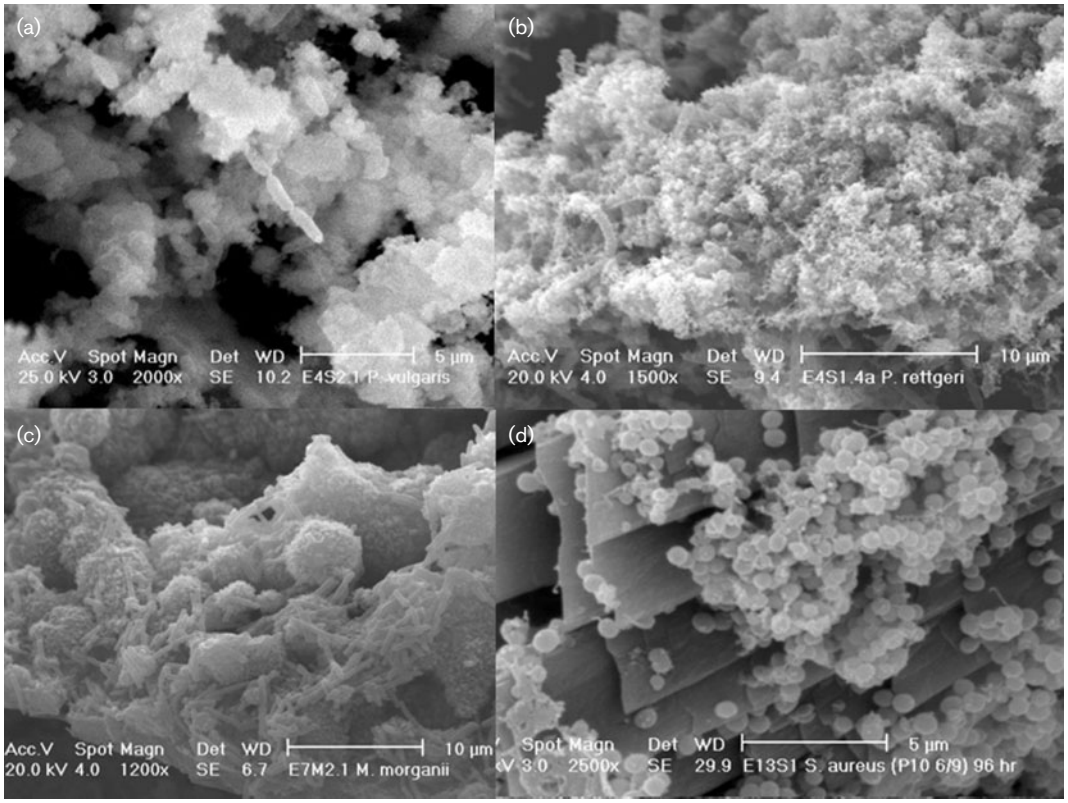

Fig. 3. Scanning electron micrographs of crystalline biofilms. (a) *P. vulgaris* SDM2 biofilm at catheter blockage; (b) *Pv. rettgeri* SDM1 biofilm at catheter blockage; (c) *M. morganii* RB15 biofilm on a catheter that was still draining after 96 h; and (d) urease-positive *Staph. aureus* P10 6/9 biofilm on a catheter removed from a model after 96 h.

The biocide was shown to diffuse slowly through the balloon membrane, reducing the urinary bacterial populations, preventing the rise in urinary pH and inhibiting catheter encrustation (Stickler *et al.*, 2003). In view of these results, the susceptibilities of isolates of *P. vulgaris* and *Pv. rettgeri* from catheter-associated urinary tract infections to triclosan were tested. The results presented in Table 4 show that whereas *P. vulgaris* is fully sensitive to the biocide (MICs 0.05–0.1 µg ml^{-1}), *Pv. rettgeri* is resistant to the concentrations achievable in urine (MIC 64 µg ml^{-1}).

Table 2. Rate of catheter encrustation and urease activity of a range of urinary pathogens growing in artificial urine for 4 h in shake flask culture

Mean values calculated from three replicate experiments. Isolates of *Klebsiella oxytoca*, *Serratia marcescens* and *Enterobacter cloacae* produced urease activity of <0.1 units ml^{-1}.

Organism	Rate of encrustation of Ca+Mg (µg per catheter h^{-1})	Urease activity [µmol urea hydrolysed min^{-1} (mg protein)$^{-1}$]
Proteus mirabilis RB6	260.0 (9.36)	2.34 (0.21)
Proteus vulgaris SDM2	193.1 (27.25)	2.13 (0.19)
Providencia rettgeri SDM1	178.9 (51.13)	1.85 (0.22)
Morganella morganii RB15	56.5 (15.96)	1.34 (0.09)
Staphylococcus aureus P10 6/9	11.2 (3.63)	0.28 (0.05)
Providencia stuartii RB14	13.9 (4.12)	0.10 (0.02)
Pseudomonas aeruginosa RB16	16.3 (6.19)	0.06 (0.01)
Klebsiella pneumoniae SDM17	7.2 (1.41)	0.07 (0.02)
Escherichia coli RB13 urease −ve	7.7 (1.44)	0.06 (0.02)

Table 3. Times taken for five urease-positive species to block silver, nitrofurazone and all-silicone catheters

Mean values calculated from three replicate experiments.

Organism	Mean blockage times (h) (± SE) for each type of catheter		
	All-silicone	Silver/latex	Nitrofurazone
Proteus mirabilis RB6	20.0 (2.6)	15.7 (2.0)	26.7 (3.2)
Proteus vulgaris SDM2	25.0 (2.0)	23.7 (1.3)	27.0 (2.5)
Providencia rettgeri SDM1	26.7 (7.9)	15.3 (0.9)	29.7 (6.1)
Morganella morganii RB15	149 (13.0)	>168	131.3 (12.2)
Staphylococcus aureus P10 6/9	>168	>168	>168

Subsequent testing in bladder models in which retention balloons were inflated with triclosan (3 mg ml^{-1}) revealed that while the biocide prevented catheter blockage by *P. mirabilis* and *P. vulgaris*, it had little effect on catheter encrustation by *Pv. rettgeri* (Table 4).

A previous study had shown that isolates of *M. morganii* from patients' catheters were also resistant to triclosan (MIC >100 μg ml^{-1}) and that this species could form catheter biofilms in the presence of triclosan (Jones *et al.*, 2006). As *P. mirabilis* is currently the main cause of the problem, however, the results suggest that the triclosan strategy should prevent catheter encrustation in most cases. As with any intervention with an antibacterial agent, there is the danger that the strategy will select for resistant species, e.g. *Pv. rettgeri* and *M. morganii*, or even possibly generate triclosan resistance in *P. mirabilis* (Stickler & Jones, 2008).

The prospective study of Mathur *et al.* (2005) of catheterized patients infected with *P. mirabilis* found that there was a significant (P=0.004) positive correlation between the mean pH_n of their urine and catheter lifespan. The higher the urinary pH_n and the larger the safety margin between pH_n and pH_v, the slower the rate of encrustation and the longer catheters took to block. It was also shown that the pH_n of the urine of these patients varied between individuals and from week to week in any one individual. It was suggested that if pH_n can be manipulated in order to increase the safety margin between pH_v and pH_n, a strategy for controlling catheter encrustation could be devised which does not involve intervention by antibacterial agents.

Suller *et al.* (2005) in a study of urine from healthy volunteers found that simply by increasing fluid and citrate intakes, the pH_n of urine could be elevated to values that are rarely achieved even in *P. mirabilis*-infected urine. Subsequent experiments (Stickler & Morgan, 2006) in a laboratory model infected with *P. mirabilis* confirmed that when models were supplied with an artificial dilute citrate-containing urine with a pH_n >8.3, crystalline biofilms did not form. The effect of manipulating urinary pH_n on the ability of *P. vulgaris* and *Pv. rettgeri* to encrust and block catheters was therefore examined. Artificial urine at half the basic strength, containing 0.205 g l^{-1} and 1.5 g l^{-1} citrate (pH_n of 6.9 and 8.3, respectively), was supplied to models at 1 ml min^{-1}. Models were inoculated with the test organisms and incubated for 7-day experimental

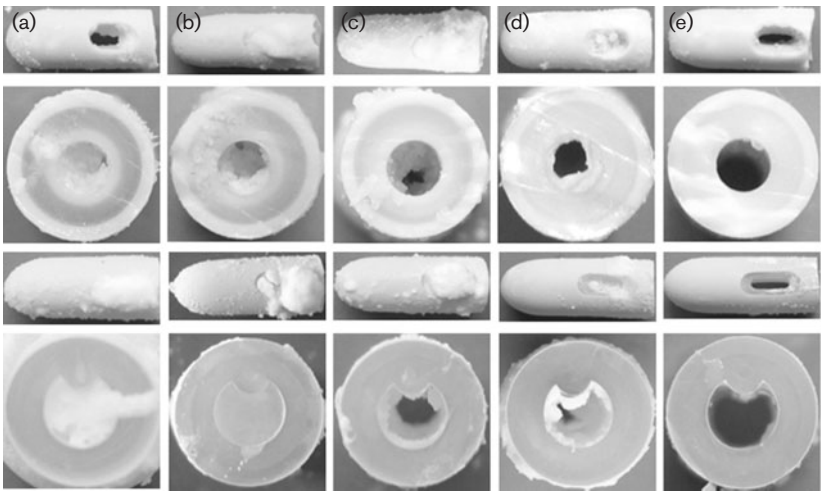

Fig. 4. Columns show the eye-holes and luminal cross-sections of antimicrobial catheters removed from models that had been inoculated with (a) *P. mirabilis* RB6, (b) *P. vulgaris* SDM2, (c) *Pv. rettgeri* SDM1, (d) *M. morganii* RB15 and (e) *Staph. aureus* P10 6/9. The catheters in (a), (b) and (c) were removed from models at blockage; those in (d) and (e) were removed after 168 h incubation. The top two rows are of silver/latex catheters; the bottom two rows show nitrofurazone/silicone catheters.

Table 4. Effect of inflating catheter balloons with triclosan (3 mg ml^{-1} in 0.1 M sodium carbonate) on the times to catheter blockage

Organism	MIC of triclosan (μg ml^{-1})	Mean times (±SE) (h) to catheter blockage*		
		Water control	0.1 M Na_2CO_3	Triclosan
Proteus mirabilis RB6	0.1	23.7 (6.9)	18.0 (1.5)	>168
Proteus vulgaris SDM2	0.1	27.0 (2.0)	38.7 (7.7)	>168
Providencia rettgeri SDM1	64	30.7 (0.3)	32.3 (6.0)	41.7 (8.5)

*Mean values calculated from three replicate experiments.

periods. The times taken for the catheters to block were recorded in four replicate experiments (Table 5). It is clear that with all three species, increasing the pH_n of urine extends the lifespan of the catheters. Scanning electron micrographs of the catheters removed after 24 h from models that had been inoculated with *Pv. rettgeri* (Figs 5 and 6) confirm that at high urinary pH_n values crystalline biofilm formation is inhibited. Similar observations were made on catheters from models inoculated with *P. vulgaris*.

The next step in the development of this strategy was to supply models with human urine from individuals who had high fluid and citrate intakes and observe the effects on catheter encrustation by *P. mirabilis*. A selection of fruit juices with high citrate contents were considered for use in the study. After tasting sessions, a drink containing a mixture of lemon and orange juice at a ratio of 1 : 4 was used. A total of 750 ml of this drink was consumed by a healthy volunteer in 250 ml aliquots at three evenly spaced intervals throughout the day. This achieved a dietary supplement of 11 g citrate. This is a modest increase in fluid intake compared to that suggested by Seltzer *et al.* (1996) as a treatment for kidney stones. These workers reported that the daily consumption of 2 l of a lemonade drink reduced kidney stone formation. A similar regimen involving the daily consumption of a drink containing 140 ml lemon juice and 250 ml summer fruit drink to aid palatability, made up to 2 l with tap water, was thus also selected for study.

The volunteer was subjected to four different fluid intake regimes each lasting a total of 3 days. In the first week, a standardized fluid intake of 1300 ml per 24 h (SFI) was consumed. In week 2, this standard intake was supplemented with 750 ml tap water (SFI + W). In week 3, the fluid supplement was 750 ml of the lemon/orange drink (SFI + C) and the final regime was SFI plus 2 l of the lemon drink (SFI + L). Urine voided was collected over 48 h on days 2 and 3. These dietary regimes were repeated over three independent periods. Intervals of 4 days were allowed between each regime. During the study, the volunteer was encouraged not to change any other aspect of diet or lifestyle. The volumes of urine collected during these periods were noted, and the pH_v and pH_n of pooled urine obtained from each regime were determined (Table 6). Urine was sterilized by filtration and then supplied to bladder models that were inoculated with *P. mirabilis* NSM6. The models were run until catheters blocked, the times to blockage being recorded (Table 6). The differences in the mean times to blockage of catheters in models supplied with urine collected during the SFI, SFI + W and SFI + C regimes were not significantly different ($P > 0.05$). The mean lifespan of catheters in models supplied with

Table 5. Effect of urinary pH_n on the blockage of catheters by *P. mirabilis*, *P. vulgaris* and *Pv. rettgeri*

Means calculated from the results of four replicate experiments.

Test organism	Mean times (h) to blockage ± SE	
	Urinary pH_n 6.9	Urinary pH_n 8.3
Proteus mirabilis	45.5 ± 1.0	>168
Proteus vulgaris	47.4 ± 2.2	>168
Providencia rettgeri	54.5 ± 2.9	>168

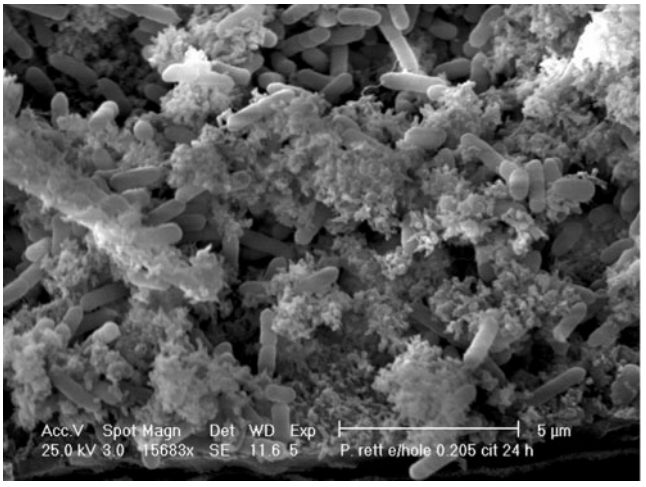

Fig. 5. Scanning electron micrograph of a *Pv. rettgeri* SDM1 biofilm removed from a bladder model being supplied with urine having a pH_n of 6.9. Microcrystalline aggregates typical of apatite can be seen associated with the bacilli.

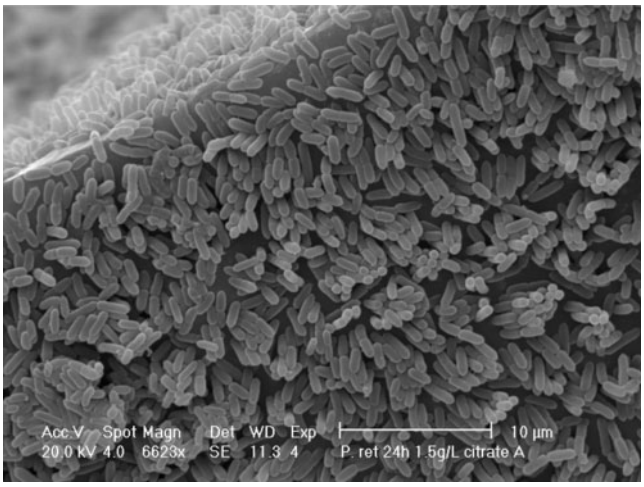

Fig. 6. Scanning electron micrograph of a *Pv. rettgeri* SDM1 biofilm removed from a bladder model being supplied with urine having a pH_n of 8.3. It is clear that no crystalline material was deposited in the bacterial biofilm.

urine collected while the volunteer was on the lemonade dietary supplement was, however, significantly longer than those from models supplied with urine from the other three regimes ($P < 0.05$). Analysis of the data obtained for each of the 12 catheters used in this experiment showed a significant correlation between log pH_n and log time to blockage (Pearson's correlation coefficient 0.844, $P < 0.001$).

A study of the problem of catheter encrustation and blockage in spine-injured patients led Burr & Nuseibeh (1997) to conclude that a high and uniform rate of fluid intake should be mandatory for a patient with a tendency to recurrent catheter blockage. Experimental evidence provided by Suller *et al.* (2005) and Stickler & Morgan (2006) has also demonstrated that dilution of urine will elevate its pH_n and slow the rate of catheter encrustation. Citrate is a natural chelating agent for divalent metal ions such as Ca^{2+} and Mg^{2+}. *In vitro* studies have confirmed that increasing the citrate content of urine will inhibit the crystallization of calcium and magnesium phosphates (Wang *et al.*, 1994), elevate the urinary pH_n (Suller *et al.*, 2005) and inhibit the encrustation of catheters by *P. mirabilis* (Stickler & Morgan, 2006). The current data show that elevating the urinary pH_n will inhibit catheter encrustation by the range of urease-positive species that are capable of producing extensive crystalline biofilms.

It is now necessary to establish the acceptability of the increased fluid regime to patients undergoing long-term catheterization and to determine whether it succeeds in elevating the safety margin between pH_v and pH_n and increasing the lifespan of catheters in patients prone to the encrustation problem. Another important issue is whether the strategy will be possible to implement and effective in the long term. Only experience will answer these questions. It is interesting, however, that Kang *et al.* (2007) reported no adverse effects in patients who had been consuming 2 l of the lemonade drink daily for up to 8 years to control kidney stones.

In conclusion, it is clear that there is strong experimental evidence and a sound physiological and physical chemical basis for the conclusion that increasing patients' fluid intake with citrate-containing drinks will be an effective strategy for the control of catheter encrustation whichever bacterial species is causing the problem. A strategy which does not involve antibacterial agents and is effective against the spectrum of urease-positive organisms capable of causing the condition has the considerable advantage that the selection of resistant organisms will not be a problem. Bacteria have developed many ingenious ways of overcoming the challenge of antimicrobial agents but it is difficult to imagine that even they can do anything about the laws of physical chemistry that govern the solubility of calcium and magnesium phosphates in urine. A clinical trial is now required to examine the effect of this manipulation of patients' urinary pH_n on catheter blockage, a complication that undermines the health and quality of life of so many elderly and disabled people.

Table 6. Characteristics of the human urine collected during the four fluid regimes

SFI, standard fluid intake; W, additional 750 ml water per 24 h; C, 750 ml lemon/orange drink per 24 h; L, 2 l lemon drink per 24 h. The values quoted are means (SE) calculated from three replicate experiments.

Property	Fluid intake regime			
	SFI	SFI+W	SFI+C	SFI+L
Urine output ml per 48 h	3633 (90)	4116 (160)	4833 (320)	7433 (230)
pH_v	5.91 (0.26)	6.1 (0.21)	6.77 (0.05)	6.29 (0.35)
pH_n	7.27 (0.150	7.7 (0.09)	7.86 (0.19)	8.1 (0.17)
pH_n–pH_v	1.36 (0.09)	1.61 (0.22)	1.1 (0.19)	1.81 (0.17)
Time (h) to catheter blockage	25.2 (5.1)	35.6 (2.3)	44.0 (8.1)	118.1 (20.1)

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