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An effective evidence-based cleaning method for the safe reuse of intermittent urinary catheters: In vitro testing

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Abstract

Aims: To determine a safe bactericidal cleaning method that does not damage urethral catheters used for intermittent catheterization. In some countries, single-use catheters are the norm; in others, the reuse of catheters is common depending on health insurance, personal preference, or individual concerns about the environment. However, no recent study of cleaning methods has been published to provide evidence for the safe reuse of catheters.

Methods: Using advanced microbiological methods, a laboratory study of eight cleaning methods was conducted. Sections of uncoated polyvinylchloride (PVC) catheters were exposed to bacterial uropathogens in physiologically correct artificial urine media then tested with a range of heat, chemical, and mechanical cleaning methods. Analysis of culturable and viable but nonculturable (VBNC) bacteria was done and direct microscopy was used. Descriptive statistics were used to compare values.

Results: Heat treatments, although effective, resulted in catheter surface breakdown and damage. Ultrasonic cleaning and vinegar showed evidence of VBNC populations indicating the methods were bacteriostatic. Detergent and water wash followed by immersion in a commercially available 0.6% sodium hypochlorite solution and 16.5% sodium chloride (diluted Milton) gave consistent bactericidal results and no visible catheter damage.

Conclusions: Combined mechanical and chemical treatment of a detergent and water wash followed by immersion in diluted Milton (the "Milton Method") provided consistent and effective cleaning of uncoated PVC catheters, showing bactericidal action for all uropathogens tested after repeated exposure. If found safe in clinical testing, this method could increase the reuse of catheters, reduce plastic waste in the environment, reduce cost, and increase patient choice.

KEYWORDS

cleaning, decontamination, intermittent catheterization, patient choice, reuse, sodium hypochlorite, uropathogens, viable but nonculturable (VBNC)

1 | INTRODUCTION

Intermittent catheterization is a common self-care procedure for bladder emptying if normal voiding is not possible. About 50,000 people in the UK use intermittent self-catheterization (ISC).[1] It is usually performed as a clean (rather than aseptic/sterile) technique by children and adults, or their family or personal carer. In the UK disposable catheters for "single use only" are used almost exclusively. This contrasts with other developed countries where catheters are commonly cleaned and reused multiple times, although cleaning methods have not been standardized.[2]

Suggested advantages of reuse include personal choice and convenience for travel, reduction in cost, and concern about waste and the environment.[3] Disadvantages include increased patient burden of cleaning, but the overwhelming concern is that reuse may increase risk of urinary tract infection (UTI) causing reuse to be understandably discouraged by many clinicians.[4] However, clinical trials have not found robust evidence that sterile single-use catheters result in better long-term outcomes in terms of reducing UTI.[4] The lack of evidence-based cleaning methods has made such studies difficult to conduct. The purpose of this study, therefore, was to investigate a range of previously reported and easily available cleaning methods to provide such evidence. In addition, it is important to consider that ISC users often have bacteriuria (bacteria in the urine) whichever technique they use, most likely due to the introduction of periurethral organisms into the bladder rather from the catheter itself.[5] Moreover, recent advances in next-generation sequencing indicate that a urinary microbiome exists, in contrast to previous assertions that the bladder is sterile.[6]

Various methods of decontamination have been evaluated previously including microwave,[7–10] boiling,[11] bleach,[12] detergent and water,[10,13–15] hydrogen peroxide,[12] povidone-iodine,[12,14] vinegar,[14] and storage in alcohol.[9] Each method demonstrated some efficacy but all the studies had limitations including the use of microbiological techniques limited to reliance on culture analysis only and/or use of nutrient broth rather than artificial urine media. The nutrient broth is a common medium in laboratories for assessing bacterial growth and attachment. However, it does not reflect the conditions found within the urinary system and bacteria may behave very differently in this environment. The use of physiologically correct artificial urine media provides a reproducible and more realistic environment to replicate bacterial survival in, and on, urinary catheters.

Previous studies have used culture analysis as the standard method to assess for the presence of bacteria. However, the use of culture-based techniques can lead to an underestimation of bacterial numbers. It is known that many bacteria can enter a viable but nonculturable (VBNC) state where metabolism is slowed, allowing them to survive when nutrients are limited or under stressful conditions.[16] The clinical impact of VBNC bacterial populations is not fully understood but it is important to understand whether any treatment is bactericidal or only bacteriostatic to determine its efficacy and safety.

It is also important that the treatment does not deleteriously affect the catheter material. One earlier study on decontamination using microwave or 70% alcohol[9] directly assessed catheter material damage by using scanning calorimetry. It concluded there was minimal change to the surface of a polyvinylchloride (PVC) catheter. In contrast, others, using microwave energy, referred to visual changes in flexibility but did not investigate further.[17]

In the literature, the most commonly cited methods of decontaminating intermittent catheters are heat-, mechanical-, or chemical-based or simply a tap water rinse. Study dates ranged from 1989 to 2009. If a safe effective method of decontamination is established using updated laboratory procedures, users of ISC would have more choice and an alternative to single-use only ISC protocols. Importantly, ISC users in countries where reuse is more common would have research evidence on which to base cleaning methods.

Thus, the purpose of this study is to determine a safe and bactericidal cleaning method for PVC catheters for potential reuse in the home situation using updated laboratory analysis methods on a range of uropathogens.

2 | MATERIALS AND METHODS

2.1 | Testing procedure

2.1.1 | Preparation of catheter samples

Sterile uncoated PVC intermittent catheters were cut into 1 to 2 cm length sections.

2.1.2 | Cleaning methods

Eight potential methods of decontamination were tested (Table 1). Methods were selected based on previous studies of cleaning catheters and other items such as feeding equipment for infants (where Milton is a commercially available product in the UK). Heat-, mechanical-, and chemical-based methods were compared against a tap water rinse control.

TABLE 1 Description of test methods used to decontaminate PVC catheter sections

Type	Treatment	Description of method
	Control	Rinsed with cold tap water.
Heat	Microwave	Using a Phillips Avent Microwave Sterilizer, 200 mL of tap water was poured into the base of the unit and catheter sections placed in the basket above. The microwave was run on full power (800 W) for 6 min. Sections were cooled for 2 min before analysis.
Heat	Boiling	Sections were placed into tap water, heated to boiling point for 2 min. They were then removed and allowed to cool.
Heat	Steam	Using a Phillips Avent three-in-one sterilizer, set up as described by the manufacturer (lower tray filled with water), sections were placed on the tray and the preset sterilization program used (4 min heating and 6 min sterilizing). Sections were removed and allowed to cool to room temperature.
Mechanical	Ultrasonic cleaning using a domestic jewelry cleaner	Sections were placed in the cleaner (JPL7000, James Products Europe Ltd, UK) with warm (45°C-50°C) tap water and cleaned according to the manufacturer's instructions (180 s). On removal, they were rinsed in tap water.
Mechanical	Detergent and water	Washed with hot soapy water. 1 squirt of liquid detergent was added to 40 mL tap water, mixed and left to soak for 5 min. Rinsed with tap water.
Chemical	White vinegar	Rinsed with tap water. A 50% (vol/vol) white malt vinegar in tap water solution was prepared. Sections were soaked for 30 min and then rinsed with tap water.
Chemical	Milton sterilizing fluid	Milton concentrate (fluid or tablet) was diluted with tap water as described in the manufacturer's instructions, resulting in a 0.6% sodium hypochlorite final concentration. Left to soak for 15 min and then rinsed with tap water.
Mechanical/Chemical	Milton Method	Detergent and water treatment as described above followed by Milton sterilizing fluid method (above).

Note: All methods used commonly found domestic equipment and solutions.
Abbreviation: PVC, polyvinylchloride.

2.1.3 | Preparation of inocula

Overnight cultures of clinical strains of bacterial uropathogens (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus* and *S. epidermidis*) (Bristol Urological Institute) were prepared in tryptone soya broth (Oxoid, UK) and incubated at 37°C. Following incubation, cultures were centrifuged at 3780*g* for 10 minutes and the pellets resuspended in artificial urine media.[18,19]

2.1.4 | Efficacy of cleaning treatments

Catheter sections were placed into 10-mL artificial urine medium with the appropriate bacterial inoculum and incubated at 37°C for 5 minutes. Sections were decontaminated using the methods shown in Table 1. For each treatment and sample, two catheter sections were removed. One section was used for microbiological analyses and the other for direct microscopy. This represented time 0, with the process being repeated after 3, 6, and 24 hours. All experiments were repeated between three and five times.

3 | ANALYSIS METHODS

3.1 | Culture enumeration

Catheter sections removed for culture analysis were placed into 10 mL 1/4 strength Ringer's solution (Oxoid). Attached bacteria were removed by sonication or vortex mixing with sterile glass beads. Aliquots were plated, in triplicate, onto tryptone soya agar (Oxoid), with serial dilution in 1/4 strength Ringer's solution, and incubated at 37°C for 16 to 24 hours. The number of colony-forming units (cfu)/cm section was calculated.

3.2 | Direct viable count

Direct viable count (DVC) was used to enumerate any VBNC bacteria.[20] Using the same resuspended samples, 1 mL aliquots were added to 4-mL autoclaved distilled water and 5-mL R2 broth. The antibiotic, pipemidic acid, was added at a final concentration of 10 μg/mL. Pipemidic acid inhibits cell division, causing viable (culturable and VBNC) bacteria to elongate. Samples were then incubated at 22°C for 16 to 24 hours. Following incubation, 1 mL aliquots were

stained with LIVE/DEAD BacLight (according to the manufacturer's instructions) (Thermo Fisher Scientific, UK) and filtered onto 0.2 μm diameter pore size Nuclepore membranes (Whatman, UK). These were examined under oil using a Nikon Eclipse LV100D epifluorescence microscope (Best Scientific, UK). Elongated cells in up to 100 randomly selected fields of view (fov) were counted.

3.3 | Evaluation of catheter surfaces and evidence of bacterial attachment

To assess surface damage, sections were examined directly under episcopic differential interference contrast (EDIC) microscopy. Catheters were scanned for surface structural damage comparing sterile catheter sections, control samples exposed to each bacterium, and sections which had been exposed and then decontaminated. A Nikon Eclipse LV100D with EXPO X-Cite 120 metal halide fluorescence system and long-working metallurgical Nikon Plan Achromat objectives (Best Scientific)[21] was used.

3.4 | Statistical analysis

Descriptive statistics were used, with mean values used for culture analysis results and median values used for DVC data.

4 | RESULTS

A summary of the microbiological results for the various methods of decontamination is given in Figure 1.

4.1 | Control (tap water rinse)

A control treatment of a tap water rinse was used as a comparative method. Following exposure to *E. coli* (Figure 1A), consistently high values were obtained for culturable bacteria greater than 5×10^2-10^4 cfu/cm catheter. When exposed to the higher concentration inoculum of *K. pneumoniae* (Figure 1B), which represented a worst case UTI scenario, between 10^6 and 10^7 cfu/cm were detected at all time points. This indicates that between 0.01% and 1% of the suspended bacterial population in the urine will attach to the catheter surface if no method of decontamination is used.

Using DVC, the numbers of culturable and dormant VBNC bacteria could be assessed (Figure 1C). Viable cells elongated clearly and could be visualized following labeling. Following the control treatment, high-median values were found and 100% of fov contained elongated bacteria, indicating the presence of metabolically active bacterial populations (Figure 1C). *K. pneumoniae* showed no difference between culturable numbers and DVC counts, therefore, the bacterial population was not sublethally stressed while in the artificial urine media.

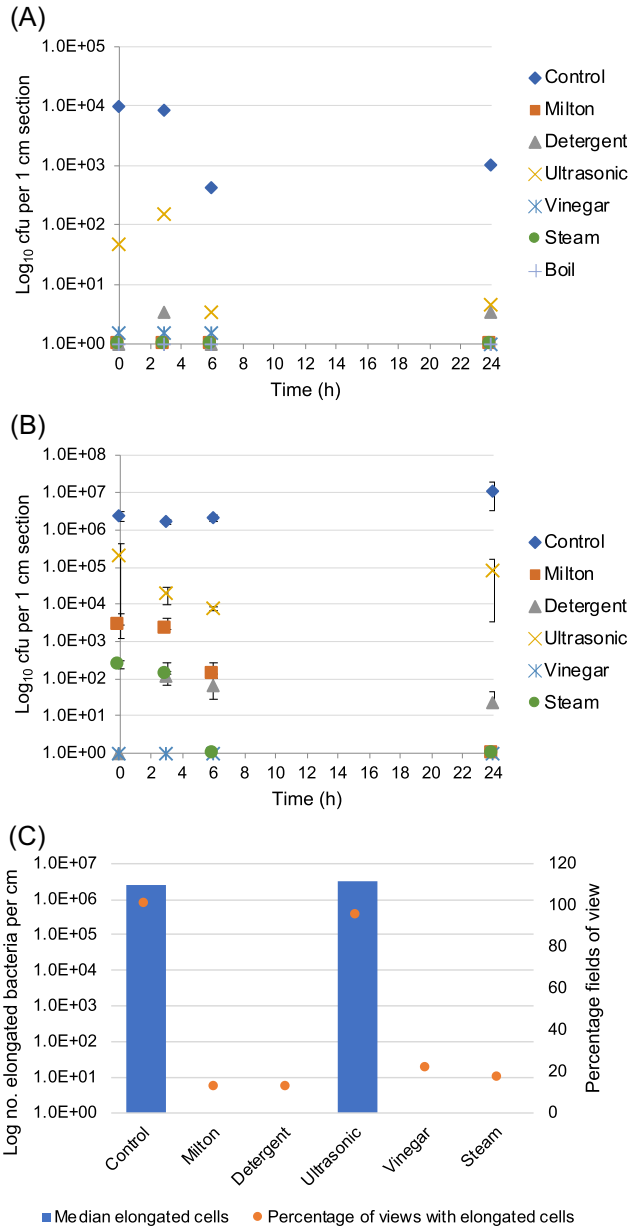

FIGURE 1 Summary of microbiological data following testing with a range of methods of decontamination over repeated exposure events in a 24 hour time period. A, *Escherichia coli* (inoculum concentration, 10^6 bacteria/mL artificial urine medium). B, *Klebsiella pneumoniae* (inoculum concentration, 10^9 bacteria/mL artificial urine medium). Mean values plotted with standard errors. C, Numbers of elongated *K. pneumonia* after repeated exposure in 24 hours (four contamination/cleaning events), median values plotted along with the percentage of fields of view containing elongated bacteria. Note: values plotted as 1 are nominal values to represent data that was below the detectable limit

4.2 | Heat-based methods

Three heat-based cleaning methods were used: microwave treatment, boiling, and steam sterilization. Microwave treatment was not fully tested due to early indications of melting and noticeable changes to flexibility of the catheters. Boiling resulted in no culturable *E. coli* but did cause some catheter flexibility changes and was not tested further. Steam sterilization was effective, resulting in no detectable culturable *E. coli* (Figure 1A) and following exposure to the high concentration inoculum of *K. pneumoniae*, values decreased from only 10^2 cfu/cm catheter to undetectable. When considering DVC analysis, the median values for *K. pneumoniae* (Figure 1C) were zero but 17% of the fov did contain elongated bacteria demonstrating some development of a VBNC population, although low compared to the control.

4.3 | Mechanical methods

Two mechanical methods were tested: an ultrasonic jewelry cleaner and detergent and water. The ultrasonic cleaner gave the lowest reduction in culturable bacteria (Figure 1A,B), only approximately 2-log lower than the control. This was also observed following DVC (Figure 1C; with high median and percentage fov data), giving values directly comparable to the control). The difference between the culturable bacterial numbers and the DVC counts indicates the presence of the VBNC population, with the cells being sublethally damaged/stressed by the action of the ultrasonic cleaner.

Following cleaning with detergent and water, the numbers of culturable *E. coli* were less than 10^1 cfu/cm (Figure 1A) and for *K. pneumoniae* decreased from 10^2 to 10^1 cfu/cm (Figure 1B). When tested with the DVC technique, median values were zero (Figure 1C) with 12% of fov containing elongated cells.

4.4 | Chemical methods

Two chemical treatments were tested: vinegar and diluted Milton (0.6 sodium hypochlorite/16.5% sodium chloride). Following treatment with vinegar, less than 10^1 cfu/cm culturable *E. coli* were detected (Figure 1A) and no detectable culturable *K. pneumoniae* (Figure1B) (a 6-7-log reduction compared to the control). DVC analysis for *K. pneumoniae* (Figure 1C) indicated median values were zero with 21% of the assessed fov contained at least one elongating bacterium, giving evidence of some development of a VBNC population.

Immersion in diluted Milton resulted in no culturable *E. coli* detected at any time point (Figure 1A). For the more concentrated *K. pneumoniae*, 10^3 bacteria/cm culturable were detected at times 0 and 3 hours, decreasing to 10^2 after 6 hours and undetectable at 24 hours (Figure 1B). Median values, following DVC, were zero in all cases, with percentage fov containing at least one elongated bacterium being 12% for *K. pneumoniae* (Figure 1C). This value was comparable to cleaning with detergent and water, and indicates a low-level development of a VBNC population.

When tested with the full range of uropathogens, it was found that the use of diluted Milton consistently resulted in no detectable culturable bacteria after repeated exposure over 24 hours (Figure 2). This is important as it demonstrates efficacy against both Gram-negative and Gram-positive bacteria.

4.5 | Combined method

For other uses, a detergent and water prewash is recommended before immersion in diluted Milton (manufacturer's instructions). *E. coli* was used to establish whether this combined procedure (the "Milton Method") was more effective than either diluted Milton or detergent and water alone. A high inoculum concentration (10^9 cells/mL) was used, along with a tap water control.

The DVC method was used to assess for VBNC populations with results shown in Table 2. Median values are given with the control samples having a value of 4.9×10^6 cells/cm per catheter. The control samples had 96% of fov containing elongated bacteria. Median values for all cleaning treatments were zero. However, when the percentage fov containing one or more elongated *E. coli* was calculated, 44% contained elongated cells following

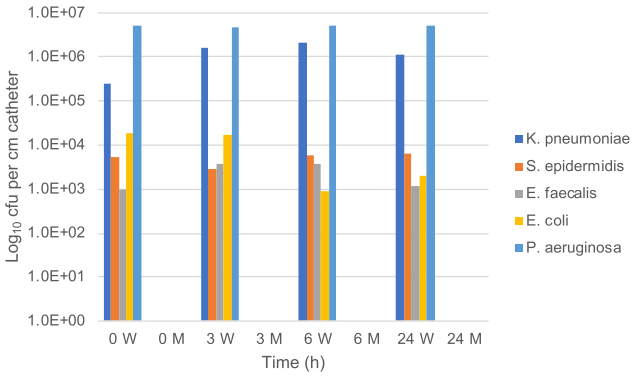

FIGURE 2 Culture analysis data comparing a tap water rinse control against the use of Milton solution, after repeated contamination and decontamination events. The uropathogens *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa.* M, treatment with Milton solution; W, tap water rinse

TABLE 2 Summary of direct viable count (median elongated bacteria/cm length catheter) results for *E. coli* following a control treatment of a tap water rinse, with detergent and water only, Milton solution only, and the combined "Milton Method"

	Control	Detergent	Milton	"Milton method"
Median values (number of elongated bacteria per 1 cm catheter section)	4.9×10^6	0	0	0
% Fields of view with elongated cells	96	44	38	0

Note: The percentage of fields of view with visible elongated bacteria is also given.

detergent and water and 38% after soaking in diluted Milton. These values are higher than observed for *K. pneumoniae*, demonstrating the adaptations and increased ability to persist in the urinary tract displayed by uropathogenic *E. coli*. In contrast, the combination of cleaning in detergent and water rinse followed by immersion in diluted Milton gave no positive results, with no visible elongated cells in any fov. This indicates that there was no development of a VBNC population and the "Milton Method" is fully bactericidal.

5 | CATHETER ASSESSMENTS USING EDIC MICROSCOPY

EDIC microscopy was used to examine catheter surfaces before and following repeated exposure to decontamination treatments and bacteria. The unused catheter surface was highly disordered. Damage and changes to surfaces were observed in some samples (Figure 3).

The use of microwave energy caused macroscopic catheter damage and changed the flexibility of the catheter. For this reason, it was abandoned early during testing. Following repeated boiling or steam sterilization, catheters became less flexible or had areas of surface damage (Figure 3E,F). The ultrasonic jewelry cleaner also caused areas of surface degradation (Figure 3D). Chemical methods (diluted Milton or vinegar) or detergent and water did not affect the surface structure (data not shown).

Following bacterial contamination, control samples showed clear attachment of bacteria (Figure 3A). The deposition of bacteria was also observed following steam and ultrasound treatment (Figures 3C and 3F). When decontaminated with steam, large areas of bacterial attachment were found (Figure 3F), the action of the steam fixing the bacteria to the catheters.

6 | DISCUSSION

Using extensive multimethod laboratory testing and a range of conventional and novel analysis procedures, we evaluated several methods of decontaminating PVC catheters for reuse. Of the methods selected, the "Milton Method" gave consistently effective results over repeated reuses. The "Milton Method" consists of a detergent and water wash followed by a 15 minutes immersion in diluted Milton. Milton is a well-known household brand in the UK, used for sterilization of baby feeding equipment and other domestic applications. This solution consists of 0.6% sodium hypochlorite and retains activity over 24 hours before free chlorine levels decrease.

Compared to earlier studies, our results are strengthened by three key factors:

1) Improved analysis methods—use of culture and DVC to give assessments of bactericidal vs bacteriostatic effects of the various methods of decontamination.
2) Use of two artificial urine media—one representing "healthy" urine[18] and the other "diseased" urine[19] to show that the method of decontamination is effective regardless of variations in urine composition.
3) Analysis of catheter damage—use of EDIC microscopy[21] to examine all areas of a range of commercially available uncoated PVC catheters to assess for damage or surface structural changes.

Previous studies have relied solely on culture analysis. Although culture analysis is widely used to assess bacterial viability, there is evidence demonstrating the presence of VBNC populations.[22–24] VBNC bacteria are sublethally stressed and may have the ability to cause infection when conditions improve.[21–23] By using DVC and comparing it with standard culture techniques, we demonstrated how the "Milton Method" did not lead to the development of VBNC populations. It, therefore, had bactericidal rather than bacteriostatic activity. In contrast, while both stages of the "Milton Method" (detergent and water rinse or immersion in diluted Milton only) gave good results by culture analysis, individually they did result in low levels of VBNC population development. Other methods such as the ultrasonic jewelry cleaner had high levels of VBNC populations present. The 2-log reduction observed by culture recovery (compared to the control samples) implies some effect from the cleaning process; however, the DVC values remained comparable to the control, thus indicating a bacteriostatic effect only. In control samples (tap water rinse), no differences were observed between culture and DVC analyses. This indicates that the bacteria were all in an active metabolic state in the artificial urine medium. This is important as it shows how

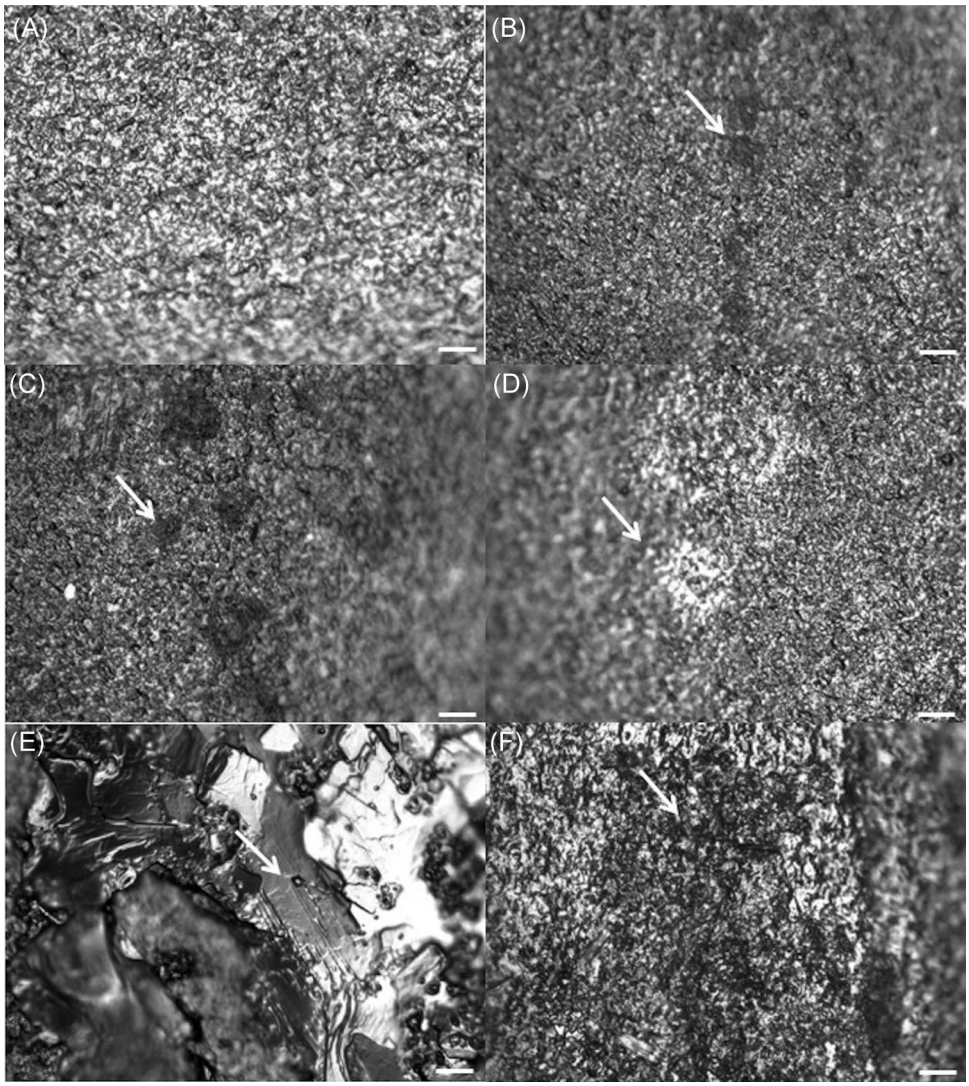

FIGURE 3 Episcopic differential interference contrast microscopy images of uncoated PVC catheters pre- and postbacterial exposure and subsequent decontamination. A, Unused PVC catheter surface before bacterial exposure and decontamination. B, Tap water control following two contamination events at 0 and 3 hours. Evidence of bacterial attachment indicated by arrows. C and D, Ultrasonic cleaning after 3 hours (C) and 6 hours (D), representing two and three contamination and decontamination events. Arrows indicate evidence of bacterial attachment (C) and surface damage (D). E and F, Steam sterilization treatment after 24 hours, representing four contamination and decontamination events. Clear evidence of gross material breakdown (E) and bacterial deposition (F) indicated by arrows. Magnification, ×500 and scale bar = 20 μm. PVC, polyvinylchloride

the environment did not cause sublethally damaged/stressed bacteria and any subsequent development of a VBNC population was, therefore, due to the method of decontamination.

Heat-based methods caused a number of problems and gave inconsistent results. Steam sterilization enhanced bacterial attachment when surfaces were examined directly. While the majority of the bacterial populations were killed, steam was acting as a fixative and causing the material to adhere to the catheter material. Microwave, boiling, and steam caused structural damage to some catheters. This was also found in the previous studies[9,17] where melting was observed and linked to the type of microwave used, with lower power domestic microwaves causing less widespread damage but affecting catheter rigidity. In the current study, large areas of the catheter, where the surface characteristics had been altered, were also clearly visible following steam sterilization. The flexibility of the catheters was altered and microscopic analysis showed how the polymer structure was being broken down. Thus, heat-based methods for PVC catheter cleaning are not recommended.

The use of physiologically correct artificial urine media is important. Both bacteria and chemical agents will respond differently in varying environmental conditions, and it is essential that any in vitro study reflects real conditions as accurately as possible. Previous studies, such as Kurtz et al,[12]

have used standard broth media, which could result in very different responses from the *E. coli* or indeed affect the activity of the bleach solution they tested. In this study, the use of two artificial urine media strengthens the validity of the current study, with one representing a "'healthy" urine[18] and the other, a "diseased" urine,[19] two conditions likely to be reflected in ISC users who are all likely to have asymptomatic bacteriuria but not necessarily UTIs.

7 | CONCLUSION

In this paper, we have presented data on a range of methods for cleaning and decontaminating catheters used for intermittent catheterization. The study has advanced previous work in this field by the use of high-concentration bacterial uropathogens, appropriate artificial urine media and analysis, and advanced microscopy. We demonstrated that the combined mechanical (detergent and water) and chemical (Milton; 0.6 sodium hypochlorite/16.5% sodium chloride) "Milton Method" was an effective, consistent, and simple procedure in the laboratory for repeated decontamination of uncoated PVC catheters which does not cause surface damage. This approach provides a superior cleaning treatment that is bactericidal against a range of commonly found uropathogens. The safety and suitability of this method for use by catheter users now requires clinical testing. If found safe in clinical testing, this method could increase the reuse of catheters leading to reductions in plastic waste and cost while increasing patient choice.

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