Elimination of biofilm and microbial contamination reservoirs in hospital washbasin U-bends by automated cleaning and disinfection with electrochemically activated solutions

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Running title: Automated disinfection of washbasin U-bends

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Summary

Background: Washbasin U-bends are reservoirs of microbial contamination in healthcare environments. U-bends are constantly full of water and harbour microbial biofilm.

Aim: To develop an effective automated cleaning and disinfection system for U-bends using two solutions generated by electrochemical activation of brine including the disinfectant anolyte (predominantly hypochlorous acid) and catholyte (predominantly sodium hydroxide) with detergent properties.

Methods: Initially three washbasin U-bends were manually filled with catholyte followed by anolyte for five min each once weekly for five weeks. A programmable system was then developed with one washbasin that automated this process. This U-bend had three cycles of five min catholyte followed by five min anolyte treatment a week for three months. Quantitative bacterial counts from treated and control U-bends were determined on blood agar (CBA), R2A, PAS and PA agars following automated treatment and on CBA and R2A following manual treatment.

Findings: The average bacterial density from untreated U-bends throughout the study was $>1 \times 10^5$ CFU/swab on all media with *Pseudomonas aeruginosa* accounting for approximately 50% of counts. Manual U-bend ECA treatment reduced counts significantly (<100 CFU/swab) ($P < 0.01$ for CBA; $P < 0.005$ for R2A). Similarly, counts from the automated ECA-treatment U-bend were significantly reduced with average counts over 35 cycles on CBA, R2A, PAS and PA of 2.1(±4.5) ($P<0.0001$), 13.1(±30.1) ($P<0.05$), 0.7(2.8) ($P<0.001$) and 0(±0) ($P<0.05$) CFU/swab, respectively. *Pseudomonas aeruginosa* was eliminated from all treated U-bends.

Conclusion: Automated ECA treatment of washbasin U-bends consistently minimises microbial contamination.

Keywords: Washbasin U-bends, *Pseudomonas aeruginosa*, biofilm, electrochemically activated solutions, anolyte, catholyte

Introduction

Hospital water systems and associated fixtures and fittings have been identified as significant reservoirs of microbial contamination responsible for nosocomial infections, particularly among immunocompromised patients and in intensive care units (ICUs).[1-3] Microbial biofilms readily form within washbasins and sinks and their wastewater outlets and associated pipework.[4] These include the U-bend, which retains water to provide a barrier preventing sewer gas from wastewater pipes entering buildings. Furthermore, U-bends collect hair and other debris, and are frequently stagnant. U-bend biofilms can act as reservoirs and disseminators of infection by a range of bacteria, many of which harbour antimicrobial resistance elements.[1,2,5,6] Often these bacteria are motile, especially *Pseudomonas aeruginosa* and other Gram-negative species, which along with water flow, splashing and aerosolisation facilitate retro-contamination of washbasins, sinks and taps.[1,3,5,7,8]

Biofilm present in wastewater pipework is difficult to eradicate by conventional disinfection. Several approaches have been investigated to reduce the microbial bioburden in hospital washbasin and sink drains including fixture replacement, regular manual disinfection and the use of thermal disinfection by installing a heating element into U-bends.[2,4,8] Fixture replacement is not effective in the long-term as new washbasins and pipework rapidly become colonised with microorganisms.[2] Disinfectants have diminished efficacy against dense biofilms present in U-bends and associated pipework, and whereas they can temporarily reduce bioburden, they must be applied regularly due to frequent water stagnation in U-bends.[2,4] Thermal disinfection of U-bends has been shown to be effective but is not in widespread use.[8]

Previously we used the pH-neutral electrochemically activated solution Ecasol as a residual disinfectant to effectively minimise microbial contamination of dental unit waterline output and washbasin tap water in long-term studies.[9-11] Electrochemically activated (ECA) solution generators produce two solutions during electrochemical activation of dilute salt solutions; an oxidant solution capable of penetrating biofilm termed anolyte such as Ecasol (predominantly hypochlorous acid ($HOCl$)) and a catholyte with detergent properties (predominantly sodium hydroxide ($NaOH$)).[9] The purpose of this study was to investigate whether automated filling of a hospital washbasin U-bend for short periods of time with catholyte as a cleaning agent followed by automated filling with anolyte as a disinfectant would be effective at eradicating biofilm and minimising microbial contamination.

Methods

Chemicals

All chemicals and reagents used were of analytical or molecular biology grade and were purchased from Sigma-Aldrich (Wicklow, Ireland).

Anolyte and catholyte solutions

Anolyte and catholyte were produced by electrochemical activation (ECA) of a 0.2% (w/v) NaCl solution using an Ultra-Lyte Mini-UL-75a ECA generator (Clarentis Technologies, Florida, USA). The generator was configured to produce anolyte with 450 ppm free available chlorine (FAC) at pH 7.0 and catholyte with 400 ppm NaOH. For U-bend treatment freshly generated anolyte and catholyte were used undiluted and diluted 1:10 with mains water, respectively.

Measurement of free available chlorine

FAC levels in anolyte were measured using a Hach Pocket Colorimeter II (Hach Company, Iowa, USA) according to the manufacturer's instructions.

Test and control washbasins

Six identical ceramic washbasins (Armitage Shanks, Staffordshire, United Kingdom) located in adjacent staff bathrooms at the Dublin Dental University Hospital were included in the pilot study. All bathrooms are in frequent use Monday-Friday. Three months prior to the study washbasins were equipped with new Multikwik polypropylene U-bends (Marley Plumbing and Drainage, Kent, United Kingdom) with a cleaning port above the U-bend water line. The washbasin wastewater outlets were located underneath the tap water flow. One test washbasin was selected for automated ECA treatment studies, with a second used as a control.

Pilot study of ECA treatment of U-bends

Preliminary experiments were undertaken with three washbasins to investigate the efficacy of ECA solutions to minimise U-bend contamination with three additional washbasins used as controls. A manual valve was fitted to the wastewater pipe downstream of each washbasin U-bend to seal the wastewater outflow. The volume of liquid required to completely fill the U-bends and the wastewater pipe as far as the valve was determined empirically. For the test washbasins the valve was closed and the required volume (approximately 1 L) of catholyte was poured slowly into the washbasin filling it several centimetres above the wastewater outlet. Then the valve was partially opened to allow catholyte to completely fill the U-bend and outflow pipe as far as the valve while ensuring that sufficient catholyte remained in the washbasin to cover the wastewater outlet. Catholyte was left in situ for five min and then the valve was opened to void the solution to waste. The process was repeated with freshly generated anolyte. The same process was repeated for the control washbasins using mains water instead of ECA solutions. An area of the internal part of the U-bends were swab sampled through the cleaning ports using swabs soaked in neutralisation solution followed by laboratory culture on blood agar and R2A agar (see below).

Automated ECA treatment system for U-bends

For automated U-bend treatment, one washbasin was used as the control unit and a second as the test unit. A lockable cabinet was installed adjacent to the test washbasin to house dosing pumps and two 10-L polypropylene reservoirs for anolyte and catholyte. Each reservoir supplied separate dosing pumps connected by 6 mm diameter polyvinylidene fluoride flexible tubing at separate points to the wastewater pipe connected below the washbasin U-bend. A 40 mm ball valve with an actuator, permitting automated valve control, were fitted to the wastewater pipework downstream from the U-bend replacing the manual valve used in preliminary experiments. The actuator and pumps was regulated by an electronic process controller, which allowed the timing, duration and sequence of activation of the actuator and pumps to be pre-programmed. The system is outlined schematically in Figure 1.

Automated treatment cycles were timed for 07.00 h and began with the actuator closing the valve on the wastewater outflow pipe. Following a 20 s delay a pump began dosing catholyte into the system from the lowest point on the pipework upstream of the U-bend. During this process, which took 5 min, catholyte slowly retro-fills the U-bend and causes air and water from the U-bend to rise into the washbasin through the wastewater outlet opening. Catholyte was left in situ for five min and then voided to waste by automated opening of the valve. Following a 20 s delay the actuator closes the valve and following a further 20 s delay a second pump doses anolyte into the system and the cycle proceeds as per catholyte dosing. Anolyte was left in situ for 5 min and then voided to waste, completing the cycle.

Microbiological culture of U-bend samples

Immediately following each of 35 ECA treatment cycles the interior surface of the U-bends from the test and control washbasins were sampled through the cleaning ports using sterile cotton wool swabs (Venturi, Transystem, Copan, Italy). In the case of 18 treatment cycles, additional samples were taken 24 h post-treatment. Swabs were dipped in sodium thiosulphate (0.5% w/v) solution before use to neutralise residual FAC and were processed immediately.[10,11] The tip of each was cut off and suspended in 1 ml of sterile water, vortexed for one min, serially diluted and 100 µl aliquots spread in duplicate onto Columbia blood agar (CBA) (Lip Diagnostic Services, Galway, Ireland), R2A agar (Lip), Pseudomonas aeruginosa selective agar (PAS) (Oxoid Ltd., Basingstoke, United Kingdom) containing cetrimide (200 µg/ml) and sodium nalidixate (15 µg/ml) and Pseudomonas selective agar (PA) (Oxoid) containing cetrimide (10 µg/ml), fusidic acid (10 µg/ml), and cephaloridine (50 µg/ml). PAS and PA agar plates were incubated at 30°C for 48 h, CBA plates were incubated at 37°C for 48 h and R2A agar plates were incubated at 20°C for 10 days. R2A agar permits the recovery of significantly more bacteria from water or aqueous environments than conventional, more nutritious culture media, at 20°C. Higher bacterial counts are recovered on R2A following prolonged incubation (i.e. 10 days) ensuring the

maximum number of bacteria are detected. The inclusion of sodium pyruvate in R2A medium also leads to enhanced recovery of chlorine stressed bacteria.[10]
Colonies were counted using a Flash and Go™ automatic colony counter (IUL Instruments Ltd., Barcelona, Spain). Results were recorded as colony forming units (CFUs) per swab. The characteristics of different colony types recovered and their relative abundance were recorded and selected colonies of each were stored at -80°C in Microbank cryovials (Prolab Diagnostics, Cheshire, United Kingdom) prior to identification.

Identification of bacterial isolates

Bacterial identification was determined by comparing small ribosomal subunit rRNA gene sequences with consensus sequences for individual bacterial species in the EMBL/GenBank databases.[9,10]

Statistical Analysis

Statistical analyses were performed using GraphPad Prism v.5 (GraphPad Software, San Diego, USA). Statistical significance was determined using an unpaired, two-tailed Student's t-test with 95% confidence interval (C.I.).

Results

Manual U-bend treatment with ECA solutions

Microbiological sampling of the three control washbasin U-bends tested once weekly for five consecutive weeks showed all were heavily contaminated. The mean average bacterial density on CBA and R2A agars was 2.41 x 10^5 (±2.5 x 10^5) and 1 x 10^6 (±9.9 x 10^5) CFU/swab, respectively, (CBA range 4.8 x 10^3 -7.6 x 10^5 CFU/swab; R2A range 9.2 x 10^3 - 3.8 x 10^6 CFU/swab). In contrast, swab samples from the three test washbasin U-bends treated with ECA solutions once weekly for five consecutive weeks showed significant reductions in bacterial density on both media relative to the untreated U-bends (CBA $P < 0.01$; R2A $P < 0.005$). The mean average density on CBA and R2A agars for the treated U-bends was 25.7(±73.9) and 48.5(±92.9) CFU/swab, respectively, (CBA range 0-290 CFU/swab; R2A range 0-340 CFU/swab). These findings indicated that U-bend contamination could be significantly reduced by completely filling U-bends with catholyte followed by anolyte for short time periods.

Automated U-bend treatment with ECA solutions

An automated system was developed enabling the U-bend of one of the test washbasins to be completely filled with catholyte followed by anolyte for set time periods followed by automated voiding to waste (Figure 1). The U-bend was subjected to three weekly treatment cycles (Monday, Wednesday and Friday) with catholyte for five min followed by anolyte for a further five min for a three-month period (35 cycles in total). Neutralised swab samples were taken following each treatment cycle and the quantitative density of bacteria recovered determined on a variety of culture media. An untreated washbasin U-bend was used as a parallel control. The average bacterial density from the control U-bend throughout the study period on CBA, R2A, PAS and PA media was in excess of 1 x 10^5 CFU/swab in each case (Table 1). In contrast, the average bacterial density from the ECA-treated U-bend on CBA, R2A, PAS and PA was 2.1(±4.5), 13.1(±30.9), 0.7(2.8) and 0(±0) CFU/swab, respectively (Table 1). For all four media the five-log reduction in bacterial density achieved between the ECA-treated and untreated U-bends was significant (Table 1). In the case of 18/35 decontamination cycles, additional U-bend samples were taken 24 h after ECA treatment, which revealed minimal contamination relative to untreated controls (Table 1). Culture analysis of neutralised swab samples taken from the interior surface of the washbasin covered by ECA solutions during automated treatment showed the absence of contamination immediately after ECA treatment (data not shown).

The bacterial species identified from different colony types cultured from the test and control U-bends throughout the study included *Comamonas testosteroni, Micrococcus luteus, P. aeruginosa*, *Pseudomonas putida*, *Staphylococcus warneri*, *Staphylococcus epidermidis*, *Stenotrophomonas maltophilia* and *Sphingomonas paucimobilis*. *Pseudomonas aeruginosa* accounted for approximately 50% of the bacterial counts recovered from control U-bend samples throughout the study and was present in 100% of samples. It was not recovered from any ECA-treated U-bend samples.

Lack of adverse effects on wastewater network

During the study, routine checks on washbasin U-bend and wastewater pipework showed no adverse affects. No leaks or corrosion were observed on pipework, pumps, valves or other components.

Discussion

Washbasin and sink U-bends are a ubiquitous reservoir of microbial contamination in healthcare environments. This study investigated whether ECA solutions could be used to minimise microbial contamination in washbasin U-bends using regular automated treatment. Because water stagnation in U-bends can result in particularly dense biofilms we harnessed the properties of both ECA solutions generated by electrochemical activation of a dilute salt solution for U-bend disinfection including the detergent properties of catholyte (containing NaOH) and the disinfectant properties of anolyte (containing HOCl). Pilot studies were undertaken with three identical test and three control washbasins with polypropylene U-bends that had a manual valve fitted on the wastewater outflow pipework enabling the U-bends to be completely filled with ECA solutions or water. The treated U-bends showed significant reductions ($P < 0.01$) in average bacterial density from between 10^5-10^6 to <100 CFU/swab.

Based on the pilot data we developed a system for automated U-bend treatment with ECA solutions. The protocol for this was the same as the pilot study except that the entire process was automated (Figure 1). Like the pilot study the average bacterial density from the control U-bend during the three month study period was $>1 \times 10^5$ CFU/swab (Table 1), whereas microbial contamination of the ECA-treated U-bend was virtually eliminated (Table 1). Furthermore, sampling of U-bends 24 h after treatment showed minimal contamination relative to controls (Table 1). The use of disinfectants such as bleach to reduce or control microbial contamination of washbasin wastewater outlets and U-bends has been previously explored. A sink flushing protocol developed by La Forgia and colleagues to control an *Acinetobacter baumannii* ICU outbreak involved regularly flushing a gallon of diluted bleach through each sink's wastewater outlet and U-bend.[2] Although effective in controlling the outbreak this approach was labour intensive and required the manual intervention of healthcare workers who had to handle large volumes of bleach, which also had to be stored on site. Our automated system does not require direct staff involvement in U-bend disinfection and ECA solutions are generated on demand. Our pilot study found that a once weekly U-bend ECA treatment regimen significantly reduced bacterial contamination to an average of 25.7(±73.9) CFU/swab on CBA. Using the automated system with three disinfection cycles weekly increased this efficacy with bacterial contamination reduced to an average of 2.1(±4.5) CFU/swab on CBA. Similar findings by Roux and co-workers using bleach to control beta-lactamase-producing-Enterobacteriaceae in sink wastewater outlets found that daily disinfection was significantly more effective than weekly.[4] A recent laboratory study suggested the use of copper pipework in sink wastewater outlets may exhibit higher antimicrobial activity than commonly used polyvinylchloride pipework.[12] However, it is unknown if the antimicrobial effect of copper would be sustained in the long term as copper can develop oxidation layers over time.

Pseudomonas aeruginosa was the most prevalent and abundant bacterial species present in untreated U-bend samples accounting for approximately 50% of counts recovered and present in 100% of untreated U-bend samples investigated in agreement with the high prevalence of *P. aeruginosa* (86.2%) detected in U-bends by Cholley *et al.*[13] In the present study *P. aeruginosa* was not detected in samples from ECA-treated U-bends. Cholley *et al.* suggested that although the daily use of bleach appeared to be an effective means of U-bend disinfection it would be prudent to assess its efficacy in the long-term. We have previously shown that ECA anolyte is a consistently effective disinfectant for minimising microbial contamination of dental unit waterlines and washbasin output water in the long term (> 2 years). In the present study we exploited the detergent/cleaning properties of catholyte and the disinfectant properties of anolyte to degrade U-bend biofilm. Neither catholyte or anolyte alone are effective at minimising microbial contamination of U-bends (data not shown). Anolyte is inactivated in the presence of organic material and by their very nature U-bends can harbour a lot of organic material.[10] Previous studies with self-disinfecting U-bends used a heating element to heat U-bend wastewater to $\geq 85^{o}$C followed by vibration cleaning was found to be effective over a 13-month study period. However, U-bend water heating activated when water temperature dropped to 75°C and when new water entered the U-bend. This could incur significant energy costs. Our automated system only requires electricity for approximately 12 min per disinfection cycle to activate the pumps and valves.

The results of this study show that complete filling of washbasin U-bends with ECA solutions can virtually eliminate microbial contamination and the system is programmable to activate when washbasins are not in use (i.e. late at night) and as frequently as desired. We are currently in the process of adapting the automated system to treat multiple washbasin U-bends as well as integrating a variety of safety measures to ensure patients or staff are not exposed to ECA solutions during treatment cycles. In our hospital, anolyte solutions have been used for several years to consistently minimise microbial contamination of water networks and taps, so no additional costs relating to acquirement of the ECA solutions were incurred.[9-11] The additional once off costs for automated U-bend treatment for up to 10 washbasin U-bends would be approximately €5,000, with annual running costs of approximately €200 and staff time requirement of about 20 minutes per week.

In conclusion, microbial contamination of washbasin U-bends can be consistently minimised by automated ECA treatment.

Acknowledgements

We thank Tom Johnson, Qlean Enterprises, LLC, Mendota Heights, Minnesota, USA for providing, installing and commissioning the Clarentis Ultra-Lyte Mini-UL-75a ECA generator used in this study.

Conflict of interest statement

None declared.

Funding sources

This study was funded by the Dublin Dental University Hospital Microbiology Unit. Pilot studies were funded by Health Research Board grant HRA_PHS/2011/2.

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Figure legend

Schematic diagram of automated washbasin U-bend treatment. Treatment cycles are initiated by the programmable process controller. At the start of each cycle the actuator closes the valve on the wastewater outflow pipe. After a 20 s delay, catholyte is pumped into the pipework below the washbasin U-bend until the pipework and U-bend are completely filled to a level a few cm above the washbasin wastewater outlet. After 5 min the valve opens and the catholyte is voided into the wastewater stream. Then the valve closes and after a 20 s delay anolyte is pumped into until the pipework and U-bend and the cycle proceeds as for catholyte dosing. After 5 min the anolyte is voided into the wastewater stream completing the cycle.

Table I

Comparative bacterial counts from a washbasin U-bend subjected to automated treatment with ECA solutions and an untreated U-bend over three months

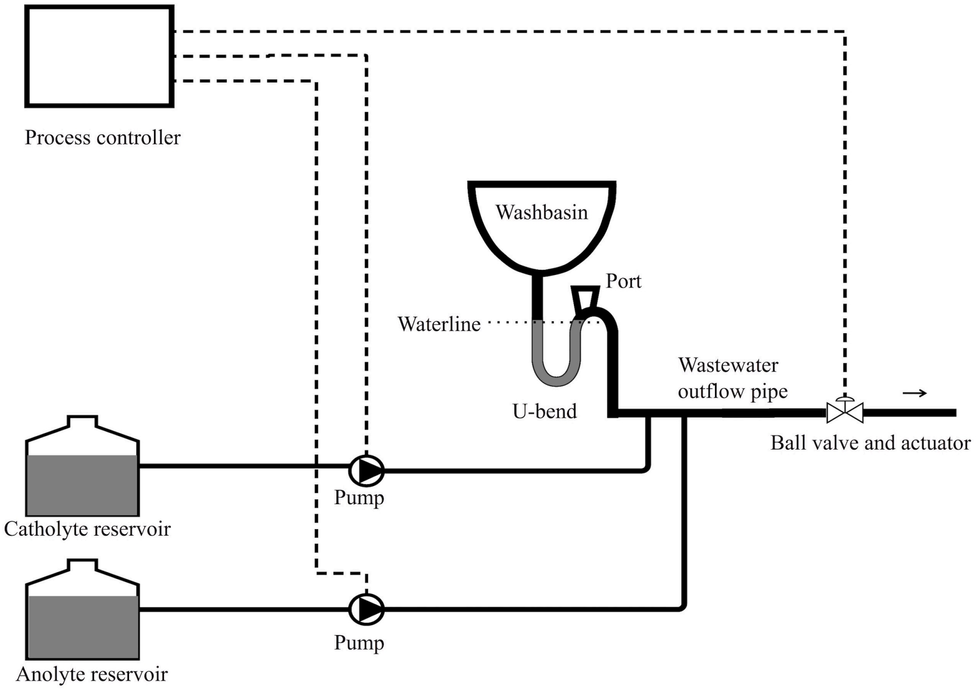
Agar medium	U-bend[a]	Average bacterial counts in CFU/swab	SD	Range of CFU/swab	*P* value
		Counts[b] immediately after treatment (n = 35)			
CBA	Treated	2.06	4.46	0-20	<0.0001
	Untreated	1.24×10^5	1.44×10^5	6.0×10^3- 7.0×10^5	
R2A	Treated	13.09	30.87	0-125	<0.05
	Untreated	3.41×10^5	8.75×10^5	3.5×10^3- 5.0×10^6	
PA	Treated	0.74	2.79	0-15	<0.001
	Untreated	1.09×10^5	1.56×10^5	2×10^3 -7.80×10^5	
PAS	Treated	0	0	0	<0.05
	Untreated	1.02×10^5	2.49×10^5	2×10^3- 1.3×10^6	
		Counts[b] 24 h after treatment (n = 18)			
CBA	Treated	35.28	83.48	0-350	<0.0009
	Untreated	1.18×10^5	1.24×10^5	9.5×10^3- 5×10^5	
R2A	Treated	82.22	199.4	0-845	<0.0075
	Untreated	1.76×10^5	2.46×10^5	7×10^3- 1×10^6	
PA	Treated	16.11	39.95	0-155	<0.0019
	Untreated	5.9×10^4	6.82×10^4	1×10^3 - 2×10^5	
PAS	Treated	13.89	33.81	0-125	<0.0093
	Untreated	3.84×10^4	5.56×10^4	1×10^3 -2×10^5	

[a] The test U-bend was subjected to 35 cycles of automated cleaning and disinfection with catholyte and anolyte over three months. Three treatment cycles were undertaken each week on Monday, Wednesday and Friday mornings after each of which the U-bend was sampled immediately with neutralised swabs. In the case of 18 of these cycles, additional samples were taken 24 h after treatment. The non-disinfected control U-bend was sampled on the same occasions.

[b] Bacterial counts were determined quantitatively.

Abbreviations: CFU, colony forming units; CBA, Columbia blood agar; R2A, R2A agar; PA, *Pseudomonas* spp. selective agar; PAS, *P. aeruginosa* selective agar; SD, standard deviation.

Figure 1


Process controller
Washbasin
Port
Waterline
U-bend
Wastewater
outflow pipe
→
Ball valve and actuator
Pump
Catholyte reservoir
Pump
Anolyte reservoir