Minimizing microbial contamination risk simultaneously from multiple hospital washbasins by automated cleaning and disinfection of U-bends with electrochemically activated solutions

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Running title: Automated decontamination of washbasin U-bends

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Summary

Background: Outbreaks of infection associated with microbial biofilm in hospital hand washbasin U-bends are increasingly being reported. In a previous study the efficacy of a prototype automated U-bend decontamination method was demonstrated for a single non-hospital pattern washbasin. It used two electrochemically-activated solutions generated from brine; a catholyte with detergent properties and anolyte with disinfectant properties.

Aim: To develop and test a large-scale automated ECA-treatment system to simultaneously decontaminate 10 hospital pattern washbasin U-bends in a busy hospital clinic.

Methods: A programmable system was developed whereby the washbasin drain outlets, U-bends and proximal wastewater pipework automatically underwent 10 min treatments each with catholyte followed by anolyte, three times weekly, over five months. Six untreated washbasins served as controls. Quantitative bacterial counts from U-bends were determined on Columbia blood agar, Reasoner's 2A agar and *Pseudomonas aeruginosa* Selective Agar following treatment and 24 h afterwards.

Findings: The average bacterial densities in CFU/swab from treated U-bends showed a >3 log reduction compared with controls and reductions were highly significant ($P < 0.0001$) on all media. There was no significant increase in average bacterial counts from treated U-bends 24 h afterwards on all media ($P > 0.1$). *Pseudomonas aeruginosa* was the most prevalent organism recovered throughout the study. Internal examination of untreated U-bends using electron microscopy showed dense biofilm extending to the washbasin drain outlet junction, whereas treated U-bends were free from biofilm.

Conclusion: Simultaneous automated treatment of multiple hospital washbasin U-bends with ECA solutions consistently minimizes microbial contamination and thus the associated infection risk.

Keywords: Washbasin U-bends, nosocomial infections, automated decontamination, electrochemically activated solutions, infection control

Introduction

Over the last two decades many studies reported hospital outbreaks, due particularly to Gram-negative bacteria, associated directly or indirectly with contaminated washbasin and sink drains [1-7]. U-bends are pieces of pipework fitted beneath washbasins that retain a volume of water creating a seal preventing sewer gas entering buildings from pipework downstream. This water may stagnate for considerable periods, encouraging the development of biofilms. These can spread as far as the washbasin drain contaminating the washbasin and surrounding area [8,9].

U-bend biofilms are usually heterogenous communities consisting of a range of opportunistic bacterial pathogens including *Pseudomonas*, *Acinetobacter*, *Klebsiella*, *and Enterobacter* spp, which can exhibit resistance to the major classes of antibiotics [2,4,6,11]. Furthermore, recent reports are increasingly highlighting the importance of wastewater pipework as a reservoir for the nosocomial transmission carbapenemase-producing *Enterobacteriaceae*, an emerging global health threat [10].

A variety of approaches to U-bend decontamination have been investigated with varying success, most of which involve disruption to service and have financial implications including the replacement of fixtures and/or associated pipework [2,6,11]. Replacement is ineffective in the long-term as new washbasins and pipework rapidly become recolonized with microorganisms. Disinfectants such as bleach may have diminished efficacy against dense biofilms, temporarily reducing bioburden but necessitating regular application [2,3,11]. Another approach involves thermal disinfection and vibrational cleaning of U-bends, but is not in widespread use [12].

Previously we showed the effective long-term use of a pH-neutral electrochemically activated (ECA) solution (anolyte) as a disinfectant to minimize microbial contamination of dental unit water and washbasin tap water [13,14]. ECA solutions are produced by passing dilute brine through an electric field in an electrolytic cell, which generates two oppositely charged solutions [13,14]. The positively charged solution (anolyte) consists of a mixture of oxidants (predominantly hypochlorous acid; HOCl), which is highly microbicidal [13]. The negatively charged antioxidant solution (catholyte) has detergent-like properties consisting predominantly of NaOH. Recently we described the development of a programmable automated prototype system for minimizing microbial contamination of a domestic pattern washbasin U-bend by treating the system sequentially with catholyte to reduce organic material followed by disinfection with anolyte [8]. Average bacterial counts from the treated U-bend over 35 decontamination cycles on a variety of culture media showed a >4 log reduction relative to controls. This pilot study established proof of concept for automated U-bend decontamination using ECA solutions.

The purpose of the present study was to develop a large-scale automated ECA treatment system capable of simultaneously decontaminating 10 hospital pattern washbasin U-bends and drains, and to robustly assess the efficacy of the system in a busy hospital clinical department.

Methods

Anolyte and catholyte

Anolyte and catholyte solutions were produced by electrochemical activation of a NaCl solution using a Qlean-Genie™ UL-75a ECA generator (Qlean Tech Enterprises, Minnesota, USA) [8]. The generator was configured to produce anolyte measured at 800 parts per million (ppm) free available chlorine (FAC) at pH 7.0, having an oxidation-reduction potential (ORP) of +880 mV and consisting of approximately 632 ppm HOCl (79%) and 162 ppm OCl^- (20.2%). Catholyte is an amphoteric surfactant with a surface tension of 63 mN force and was produced at pH 12.5 with an ORP of approximately -1000 mV, consisting of approximately 400 ppm NaOH. Freshly generated anolyte was used undiluted. FAC levels in anolyte were measured using a Hach Pocket Colorimeter II (Hach, Iowa, USA) [8]. Freshly generated catholyte was diluted 1:5 with heated mains water with a temperature after dilution of approximately 33°C.

Test and control washbasins

Ten new ceramic hospital-pattern washbasins with offset drain outlets in the back walls of the basins (Armitage Shanks, Staffordshire, United Kingdom) were installed at the A&E Department of the Dublin Dental University Hospital (DDUH) for ECA decontamination studies. Six identical washbasins located in different DDUH clinics were used as controls. Washbasins were used for hand washing only. Tork Extra Mild Liquid Soap (SCA Hygiene

Products Ltd., Bedfordshire, United Kingdom) was used for hand washing with all washbasins. Cold water supplied to test and control washbasin taps was provided from a 15,000-L tank supplied with potable quality mains water. This tank also supplied the calorifier, which provided hot water to all the washbasin taps. Automatic temperature recording was fitted on the out and return legs of the hot water network. Washbasin faucets are fitted with a thermostatic mixing valve and provided output water at an average temperature of 38ºC. Hot and cold water supplied to washbasins at DDUH has been treated with residual anolyte (2.5 ppm) for several years. Previous studies over 54 weeks showed average bacterial densities in hot and cold tap water of 1(± 4) and 2(± 4) CFU/ml, respectively [14]. All washbasins were in frequent daily use Monday to Friday. Three months prior to the study washbasins were equipped with new polypropylene U-bends (McAlpine Plumbing Products, Glasgow, Scotland) with two access ports (Figure 1).

Design of automated ECA treatment system for U-bends

A large-scale system was developed to simultaneously decontaminate 10 washbasin U-bends, drains and proximal wastewater pipework (Figure 1b). A vertical wastewater pipe below each U-bend was connected to a horizontal common wastewater collection pipe. The pipes and fittings were made of polyvinylchloride (PVC) or acrylonitrite-butadiene-styrene (ABS), both compatible with long-term exposure to anolyte and catholyte. All pipe connections apart from U-bends were chemically welded to minimize potential for leaks. ECA reservoirs were manufactured from UV-stabilized linear polyethylene designed for chemical storage. Each reservoir supplied a dosing pump (Grundfos, Bjerringbro, Denmark) connected by 25 mm ABS pipework to the common wastewater pipe (Figure 2).

A Praher unplasticized-PVC S4 ball valve (Schwertberg, Austria) was fitted to the common wastewater pipe downstream of the ECA pump connections to which an H-004 electric actuator (Actuated Solutions Ltd., Bognor Regis, United Kingdom) was fitted for automated valve operation. With the valve closed the volume of ECA solutions required to completely fill the wastewater pipework and U-bends and the washbasins to a level 5 cm above the drain outlets was determined (approximately 220 L). The timing, sequence of activation and duration of activation of the actuator-controlled valve, dosing pumps and ECA reservoir outlet valves was managed by a programmable electronic process controller (Open System Solutions Ltd., Hampshire, United Kingdom) (Figure 2).

Automated ECA decontamination cycles

Decontamination cycles began with the process controller activating the actuator and closing the valve on the common wastewater pipe. After a 30 s delay the catholyte dosing pump was activated and dosed catholyte into the common wastewater pipe and retro-filled this pipe, each washbasin's wastewater pipe, U-bend and washbasin drain outlet over a 3.5 min period. Catholyte was left *in situ* for 10 min and then voided to waste by automated opening of the valve on the common wastewater pipe. Following a further 30 s delay the actuator closed the valve and after 30 s the anolyte pump activated and dosed anolyte into the system. Anolyte was left *in situ* for 10 min and then voided to waste, completing the cycle. Control washbasin drains and U-bends were flushed with mains water instead of ECA solutions.

Microbiological culture

Decontamination efficacy was determined by semi-quantitative microbiological culture of U-bend samples (n = 620) immediately after each of 62 treatment cycles. Additional samples (n = 420) were taken 24 h post-treatment for 42 cycles to assess microbial recovery. Samples were taken from control U-bends (n = 372) following each treated U-bend decontamination cycle. U-bends were flushed with tap water after each decontamination cycle to void residual anolyte. The interior surfaces of U-bends were sampled through the access ports using sterile cotton wool swabs (Venturi, Transystem, Copan, Italy) dipped in neutralizing solution (0.5% w/v sodium thiosulphate) [8]. Six internal sites were sampled in rotation to avoid continually sampling the same parts of the U-bends (Figure 1). One site was sampled after each treatment cycle and swabs were processed immediately. The tip of each swab was cut off and vortexed for one min in one ml of sterile phosphate buffered saline, serially diluted and plated in duplicate onto Columbia blood agar (CBA) (Lip Diagnostic Services, Galway, Ireland), Reasoner's 2A (R2A) agar (Lip) and *Pseudomonas aeruginosa* Selective Agar (PAS) (Oxoid Ltd., Basingstoke, United Kingdom). PAS, CBA and R2A agar plates were incubated at 30°C for 48 h, 37°C for 48 h and 20°C for 10 days, respectively. Colony counts were recorded as CFUs per swab [8]. The characteristics of different colony types and their abundance were recorded and selected colonies of each stored [8].

Identification of bacterial isolates

Bacterial identification was determined using the Vitek MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry system (Vitek, bioMérieux Marcy l'Etoile, France) according to the manufacturer's instructions.

Electron microscopy

At the end of the study, selected U-bends were cut longitudinally and sections examined for biofilm, without prior fixation, by scanning electron microscopy [13].

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.). Statistical significance of more than two sets of data was determined using one-way ANOVA.

Results

Automated U-bend decontamination

A novel large-scale automated U-bend decontamination system was developed and installed at the DDUH A&E Department that permitted each U-bend, drain and associated wastewater pipes of 10 washbasins to be completely filled sequentially with the ECA solutions catholyte followed by anolyte (Figure 2). Empirical experiments were undertaken with the system to determine the optimal concentrations of each ECA solution for effective decontamination of the 10 U-bends in a relatively short time period. The previous proof of concept study used 450 ppm of anolyte and 40 ppm of catholyte, while for the larger system this was increased to 800 ppm anolyte and 80 ppm of catholyte. The contact time between the solutions and the pipework was increased from 5 min to 10 min. Sampling was also changed from using a single access port U-bend to U-bends with two access ports (Figure 1). This permitted six selected sites (Figure 1) to be sampled in rotation reducing mechanical removal of biofilm from repetitive sampling as ECA-treated U-bends were sampled 1040 times (Table I).

All 10 test washbasins were exposed to three weekly decontamination cycles (Monday, Wednesday and Friday) over five months (62 cycles), almost double the number of cycles assessed in the previous proof of concept study. Six additional washbasins located elsewhere in

DDUH were used as controls. Swab samples were taken from the internal surfaces of the U-bends and semi-quantitative bacterial counts were determined on CBA, R2A and PAS agar media. The average bacterial density from the six untreated U-bends during the study on CBA, R2A and PAS was 2×10^5 ($\pm 4 \times 10^5$), $3.3 \text{ x } 10^5$ ($\pm 1.1 \times 10^6$) and 2.7×10^4 ($\pm 1.2 \times 10^5$) CFU/swab, respectively, (Table I). For the 10 ECA-treated U-bends over 62 cycles, the average bacterial density on CBA, R2A and PAS was 73.4 (± 258.2), 122.5 (± 371.3) and 15.3 (± 184.5) CFU/per swab, respectively (Table I). The average reduction in viable counts from ECA-treated U-bends was >3 log or a 99.9% reduction. Reductions in average bacterial counts from treated U-bends on all media relative to the counts from control U-bends were highly significant (P <0.0001), (Table I). There was no significant difference in average bacterial counts on all media between the 10 individual treated U-bends over the study period (P >0.4). Additional U-bend samples taken from all 10 treated U-bends 24 h after treatment for 42/62 decontamination cycles showed no significant increase (P >0.1) in average bacterial counts on all media (Table I).

Bacterial species identified from U-bends

The range of bacterial species identified from treated and control U-bends throughout the study is shown in Supplemental Table S1. Although the bacterial density in treated U-bends was consistently significantly lower than controls, the diversity of species identified was greater due to a greater number of Gram-positive bacterial species comprising several species of staphylococci (Table S1). The array of Gram-negative bacterial species identified from treated and control U-bends were similar. *Pseudomonas aeruginosa* was recovered from all U-bends during the study. The average *P. aeruginosa* count from treated U-bend samples was 15 ± 185 CFU/swab (n = 620 samples), however, only 12% (74/620) of samples yielded *P. aeruginosa*, and of these only 2% yielded >10 CFU/swab. In contrast, 78% (290/372) of swab samples (n = 372) from control U-bends yielded *P. aeruginosa* and of these, 58% yielded >1000 CFU/swab.

Biofilm on ECA-treated and control U-bends

Following completion of the ECA treatment phase, the U-bends from several ECA-treated and control washbasins were removed and cut in longitudinal sections. Visual examination of the control U-bends revealed patchy, slimy biofilm on the inner surfaces, which extended to the region connecting to the washbasin drain outlet (Figure 1). In contrast, ECA-treated U-bends were visually free from biofilm (Figure 1). Electron microscopy of several sections of the inner surfaces of control U-bends confirmed the presence of dense biofilm and its absence in ECA-treated U-bends (Supplemental Figure S1).

Biofilm on washbasin drain outlet surfaces

At the end of the study period a visual examination of washbasin drain outlets revealed biofilm within the outlets of all control washbasins and its absence in treated washbasin drain outlets (Supplemental Figure 2). Neutralized swab samples taken from the drain outlets of six treated washbasins yielded average bacterial densities of 1 CFU/swab (range 0-5) on CBA agar. No bacteria were recovered on PAS agar. The corresponding average bacterial densities from control washbasin drain outlets were 4.1×10^3 (range 120- 5.6×10^3) on CBA and 874.2 (range 5- 2.7×10^3) CFU/swab on PAS. Additional swab samples were taken from the surface of each washbasin immediately adjacent to the drain outlets and no bacteria were recovered from samples from the six test washbasins on CBA or PAS media. In contrast, 3.6×10^3 (range 30- 8.6×10^3) CFU/swab was recovered on CBA and 1.2×10^3 (range 0-6.2×10^3) on PAS media from the control washbasin surface samples.

Adverse effects on washbasin wastewater network

No adverse effects were observed following regular inspection of the washbasins, U-bends or associated wastewater pipework during and at the end of the study and no leaks were identified.

Discussion

Proof of concept for effective and consistent decontamination of washbasin U-bends by automated sequential treatment with catholyte followed by anolyte was demonstrated in a previous study using a single domestic pattern washbasin located in a hospital washroom [8]. The present study developed a novel automated ECA treatment system to simultaneously decontaminate 10 hospital pattern washbasin U-bends, drain outlets and proximal wastewater pipes in a busy hospital department. The results of the study demonstrate that the large-scale system (Figure 2) has a comparable decontamination efficacy to the pilot system as both resulted in a >3 log reduction in bacterial counts in treated U-bends relative to controls (P <0.0001)

(Table I). However, with the large system >3 log reductions were simultaneously achieved in 10 separate U-bends in a busy hospital clinic, demonstrating that this approach has good potential for application in hospital departments and wards equipped with multiple washbasins. In the pilot study, *P. aeruginosa* was not recovered from the ECA-treated U-bend. The finding of low densities of *P. aeruginosa* in some ECA-treated U-bends within the larger system is not surprising because of its larger and more extensive network of pipes servicing 10 washbasins. All control and ECA-treated U-bends were positive for *P. aeruginosa* at some point during the study indicating that it is endemic within the wastewater network. Similarly, Cholley *et al.* sampled 28 U-bends over eight weeks and found that all were colonized at least once by *P. aeruginosa* [1]. In the present and in the pilot studies bacterial counts recovered immediately following ECA-treatment and 24 h afterwards were similar on all media tested (Table I), which demonstrated that biofilm within the pipework did not recover rapidly from ECA treatment [8]. A limitation to our study is that we did not demonstrate that our approach would help to control an actual hospital outbreak associated with contaminated U-bends.

A variety of Gram-negative bacterial species other than *P. aeruginosa* were identified in ECA-treated and control U-bends (Table S1). However, a greater range of Gram-positive species was identified from treated U-bends due to the recovery of several staphylococcal species not identified in the controls (Table S1). Staphylococci are common skin commensals, which inevitably get transferred into U-bends during hand washing. The recovery of staphylococci from treated U-bends, albeit in low numbers, could be due to their presence being masked by high densities of Gram-negative bacteria within the control samples.

The presence of Gram-negative bacteria in washbasin wastewater pipework constitutes a greater infection risk due to their motility. A recent study using green fluorescent protein-tagged *Escherichia coli* found that bacteria inoculated into a U-bend supplied with nutrients reached the drain outlet in a week [9]. In the present study, we found >10^3 CFU bacteria/swab within the visible biofilm in untreated washbasin drain outlets as well as on the washbasin surface in front of the outlets. In contrast, ECA-treated washbasins showed neither visible biofilm nor yielded detectable bacterial contamination within or adjacent to the drain outlets (Supplemental Figure S2). These findings show the efficacy of ECA decontamination to control biofilm within the drain outlet as well as the U-bend, impeding its ability to potentially contaminate the patient environment.

The majority of previous approaches to control hospital outbreaks linked to contaminated U-bends and drains involved pouring chemicals down the drain outlets and/or replacing the washbasin and/or associated pipework [2,3,6,11]. Vergara-López *et al.* installed manual shut off valves into sink drainage pipes followed by 30 min treatment with a quaternary ammonium compound and subsequent flushing with hot water to control a *Klebsiella oxytoca* hospital outbreak [6]. A number of valves had to be manually operated prior to manual addition of the disinfectant, which may lead to air being trapped in the pipework shielding some areas from disinfection. In contrast, the ECA decontamination system developed and tested in this study is automated and backfills the pipework from below each U-bend, reducing the likelihood of air being trapped. A recent study showed that sink-to-sink transmission can occur via a common wastewater pipe [9]. The approach used in this study minimizes opportunities for transmission of organisms between U-bends connected by common wastewater pipework as the system decontaminates drains, U-bends and pipework.

In conclusion, microbial contamination of multiple hospital washbasin U-bends and drain outlets can be consistently minimised by automated ECA treatment.

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Conflict of interest statement

None declared.

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References

1. Cholley P, Thouverez M, Floret N, Bertrand X, Talon D. The role of water fittings in intensive care rooms as reservoirs for the colonization of patients with *Pseudomonas aeruginosa*. *Intensive Care Med* 2008; **34**:1428-33. doi:10.1007/s00134-008-1110-z.

2. Hota S, Hirji Z, Stockton K, Lemieux C, Dedier H, Wolfaardt G, et al. Outbreak of multidrug-resistant *Pseudomonas aeruginosa* colonization and infection secondary to imperfect intensive care unit room design. *Infect Control Hosp Epidemiol* 2009; **30**:25-33. doi:10.1086/592700.

3. La Forgia C, Franke J, Hacek DM, Thomson RB Jr, Robicsek A, Peterson LR. Management of a multidrug-resistant *Acinetobacter baumannii* outbreak in an intensive care unit using novel environmental disinfection: a 38-month report. *Am J Infect Control* 2010; **38**:259-63. doi:10.1016/j.ajic.2009.07.012.

4. Breathnach AS, Cubbon MD, Karunaharan RN, Pope CF, Planche TD. Multidrug-resistant *Pseudomonas aeruginosa* outbreaks in two hospitals: association with contaminated hospital waste-water systems. *J Hosp Infect* 2012; **82**:19-24. doi:10.1016/j.jhin.2012.06.007.

5. Vergara-López S, Domínguez MC, Conejo MC, Pascual Á, Rodríguez-Baño J. Wastewater drainage system as an occult reservoir in a protracted clonal outbreak due to metallo-β-lactamase-producing *Klebsiella oxytoca*. *Clin Microbiol Infect* 2013; 19:E490-8. doi.org/10.1111/1469-0691.12288.

6. Leitner E, Zarfel G, Luxner J, Herzog K, Pekard-Amenitsch S, Hoenigl M, et al. Contaminated handwashing sinks as the source of a clonal outbreak of KPC-2-producing *Klebsiella oxytoca* on a hematology ward. *Antimicrob Agents Chemother* 2015; **59**:714-6. doi.org/10.1128/AAC.04306-14.

7. Chapuis A, Amoureux L, Bador J, Gavalas A, Siebor E, Chrétien ML, et al. Outbreak of extended-spectrum beta-lactamase producing *Enterobacter cloacae* with high MICs of quaternary ammonium compounds in a hematology ward associated with contaminated sinks. *Front Microbiol* 2016; **7**:1070. doi.org/10.3389/fmicb.2016.01070.

8. Swan JS, Deasy EC, Boyle MA, Russell RJ, O'Donnell MJ, Coleman DC. Elimination of biofilm and microbial contamination reservoirs in hospital washbasin U-bends by automated cleaning and disinfection with electrochemically activated solutions. *J Hosp Infect 2016*; **94**:169-74. doi.org/10.1016/j.jhin.2016.07.007.

9. Kotay S, Chai W, Guilford W, Barry K, Mathers AJ. Spread from the sink to the patient: in situ study using green fluorescent protein (GFP)-expressing *Escherichia coli* to model bacterial dispersion from hand-washing sink-trap reservoirs. *Appl Environ Microbiol* 2017; **83**:pii: e03327-16. doi.org/10.1128/AEM.03327-16.

10. Kizny Gordon AE, Mathers AJ, Cheong EYL, Gottlieb T, Kotay S, Walker AS, et al. The hospital water environment as a reservoir for carbapenem-resistant organisms causing hospital-acquired infections-a systematic review of the literature. *Clin Infect Dis* 2017; **64**:1435-44. doi.org/10.1093/cid/cix132.

11. Stjärne Aspelund A, Sjöström K, Olsson Liljequist B, Mörgelin M, Melander E, Påhlman L. Acetic acid as a decontamination method for sink drains in a nosocomial outbreak of metallo-β-lactamase-producing *Pseudomonas aeruginosa*. *J Hosp Infect* 2016; **94**:13-20. doi.org/10.1016/j.jhin.2016.05.009.

12. Fusch C, Pogorzelski D, Main C, Meyer C-L, el Helou S, Mertz D. Self-disinfecting sink drains reduce the *Pseudomonas aeruginosa* bioburden in a neonatal intensive care unit. *Acta Paediatrica* 2015; 104:e344-9. doi.org/10.1111/apa.13005.

13. O'Donnell MJ, Boyle M, Swan J, Russell RJ, Coleman DC. A centralised, automated dental hospital water quality and biofilm management system using neutral Ecasol maintains dental unit waterline output at better than potable quality: a 2-year longitudinal study. *J Dent* 2009; **37**:748-62. doi.org/10.1016/j.jdent.2009.06.001.

14. Boyle MA, O'Donnell MJ, Miller A, Russell RJ, Coleman DC. Control of bacterial contamination of washbasin taps and output water using Ecasol: a one-year study. *J Hosp Infect* 2012; **80**:288-92. doi.org/10.1016/j.jhin.2012.01.011.

TABLE I The average quantitative bacterial counts from ten washbasin U-bends subjected to automated treatment with ECA solutions and the corresponding counts from six untreated U-bends

Agar medium	U-bend	Average bacterial counts in CFU/swab from ECA-treated (n = 62 cycles, 620 swabs) and control (n = 372 swabs) U-bends	SD	Range of bacterial counts in CFU/swab	*P* value
CBA	Treated	73.4	258.2	0 - 4.6 x 10^3	<0.0001
	Untreated	2 x 10^5	4 x 10^5	0 - 4 x 10^6	
R2A	Treated	122.5	371.3	0 -5.8 x 10^3	<0.0001
	Untreated	3.3 x 10^5	1.1 x 10^6	0 – 1.8 x 10^7	
PAS	Treated	15.3	184.5	0 – 3.4 x 10^3	<0.0001
	Untreated	2.7 x 10^4	1.2 x 10^5	0 - 1.4 x 10^6	
		Average bacterial counts in CFU/swab 24 h after ECA treatment (n = 42 cycles, 420 swabs) and control (n = 252 swabs) U-bends[a]			
CBA	Treated[a]	53.2	127.6	0 – 1 x 10^3	<0.0001
	Untreated	2.1 x 10^5	4.3 x 10^5	500 – 3.2 x 10^6	
R2A	Treated[a]	91.7	277.6	0 – 3.5 x 10^3	<0.0001
	Untreated	2.9 x 10^5	6.1 x 10^5	1.3 x 10^3 – 5 x 10^6	
PAS	Treated[a]	15.6	119	0 -1.7 x 10^3	<0.0001
	Untreated	2.6 x 10^4	1.1 x 10^5	0 – 1.4 x 10^6	

[a] The average bacterial counts in CFU/swab were determined for the 10 ECA-treated U-bends and the 6 untreated U-bends 24 h after treatment for 42/62 ECA treatment cycles.

Abbreviations: ECA, electrochemically activated solution; CBA, Columbia blood agar; R2A, Reasoner's 2A agar; PAS, *P. aeruginosa* selective agar; SD, standard deviation.

Figure 1

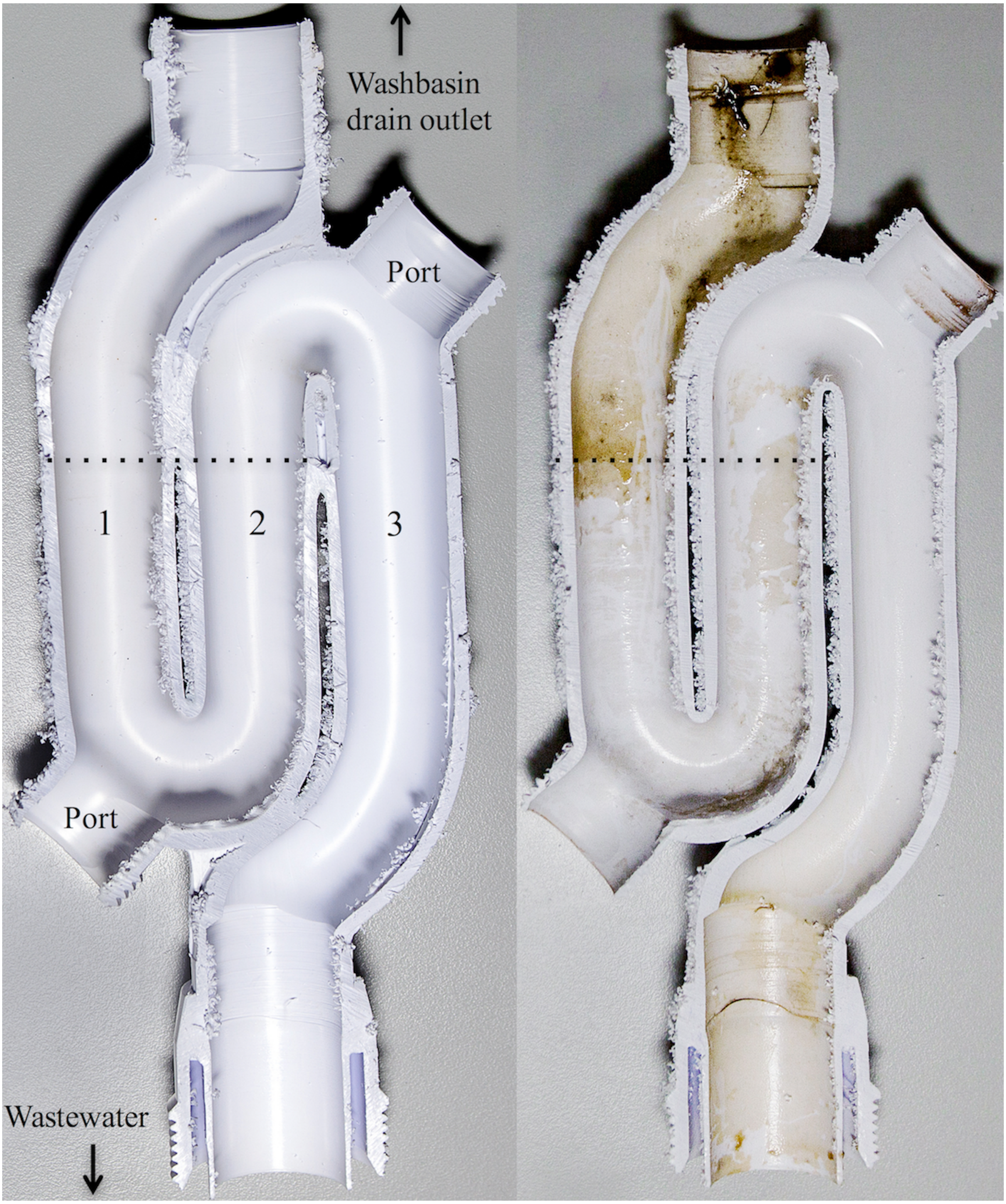


Figure 2

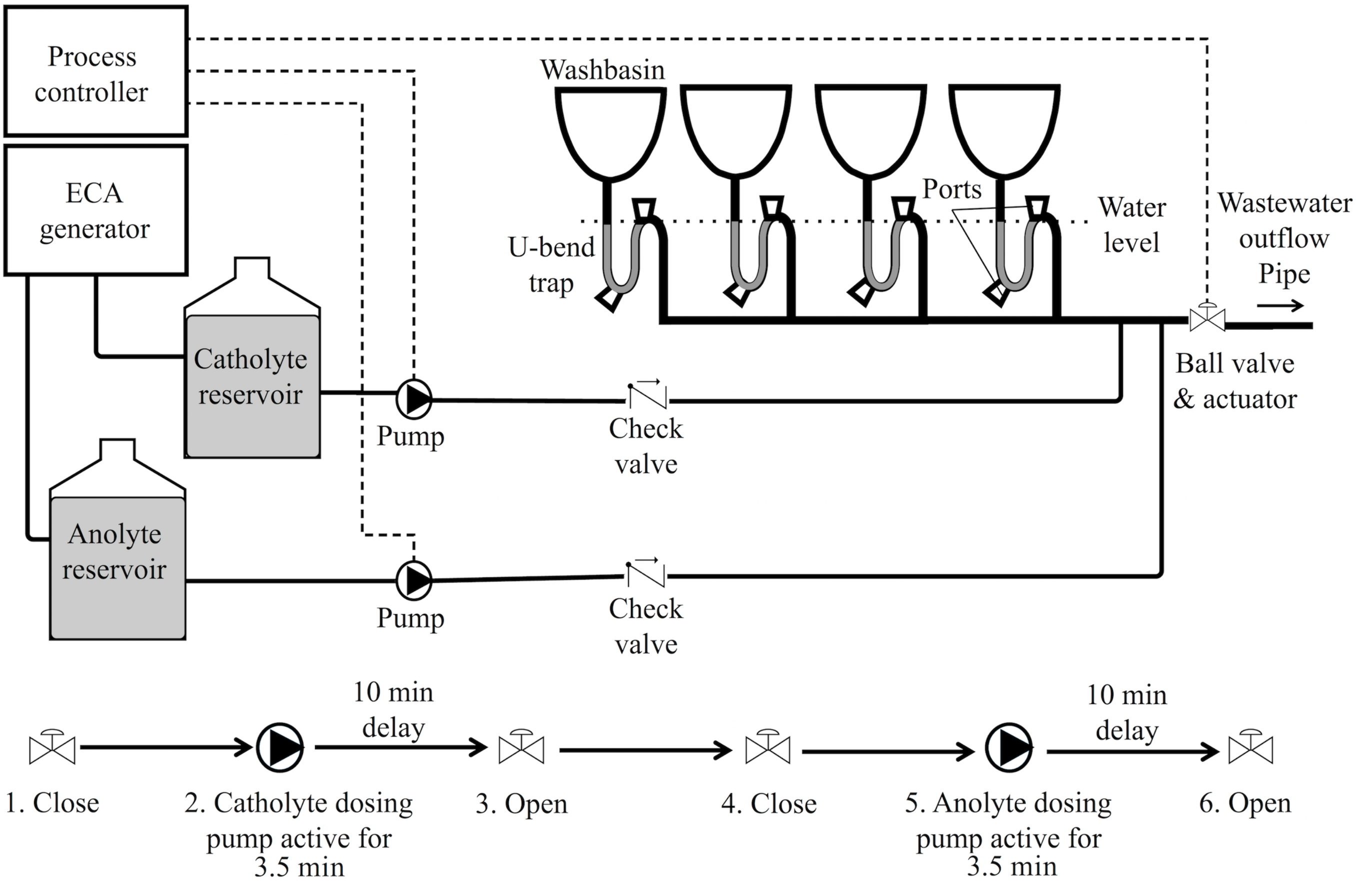

Process controller
ECA generator
Catholyte reservoir
Anolyte reservoir
Pump
Pump
Check valve
Check valve
Washbasin
U-bend trap
Ports
Water level
Wastewater outflow Pipe
Ball valve & actuator
10 min delay
10 min delay
1. Close
2. Catholyte dosing pump active for 3.5 min
3. Open
4. Close
5. Anolyte dosing pump active for 3.5 min
6. Open

Figure legend

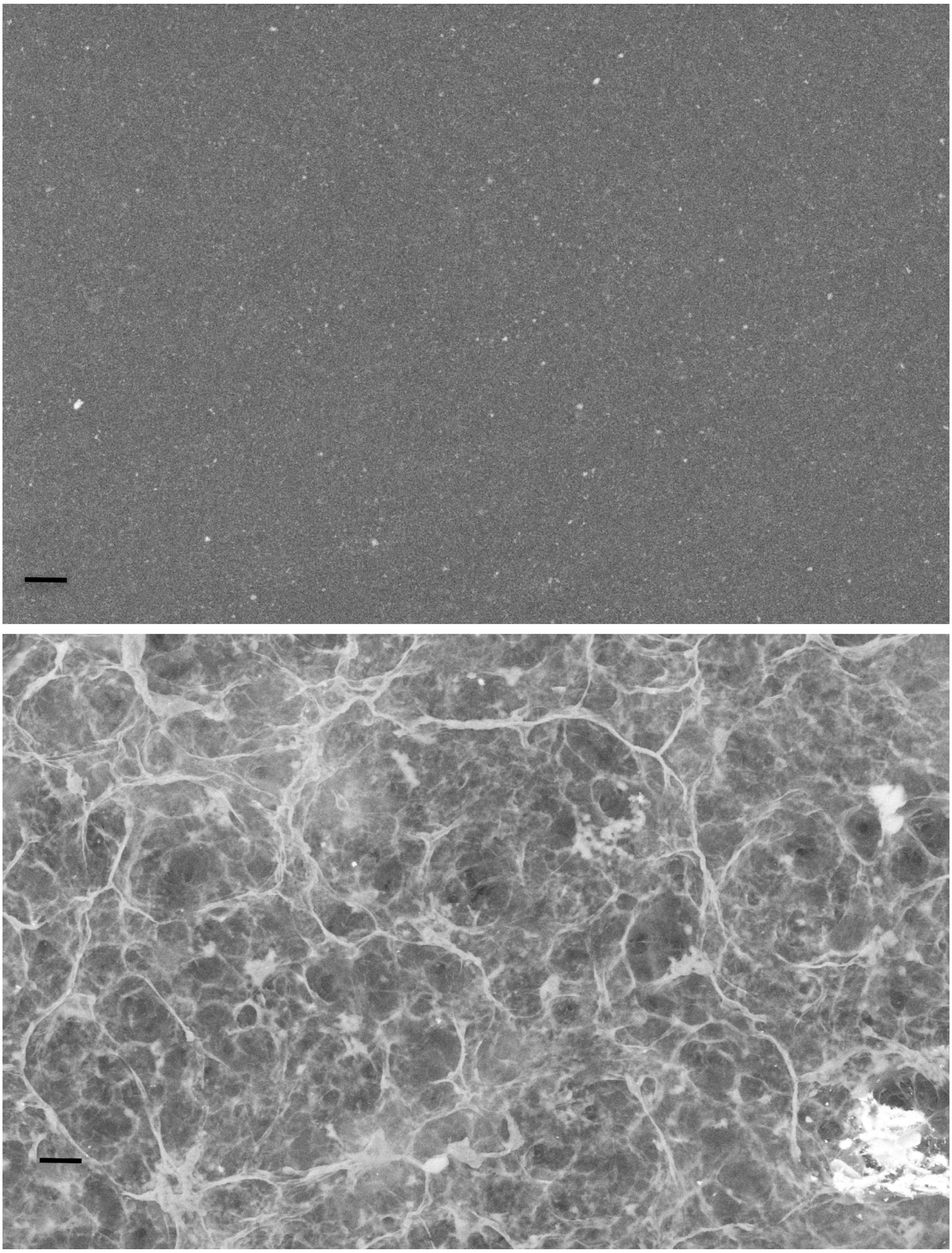
Figure 1. The left panel shows a longtitudinal section of a U-bend following 62 cycles of ECA treatment over a five-month period. The right panel shows a longtitudinal section of a control U-bend at the end of the study. Both U-bends were installed at the same time. The dashed lines indicate the water level within the U-bends. Following each ECA treatment cycle, treated and control U-bends were swab sampled through the ports indicated. To avoid continually sampling the same part of each U-bend, six internal sampling sites were selected and sampled in rotation. Three of these (labelled 1-3) are shown in the left panel. The additional three sites were located on the other, mirror image half of the U-bend. The treated U-bend is noticeably free from visible biofilm, whereas the control U-bend contains slimy biofilm, especially above the waterline and at the junctions connecting to the washbasin drain outlet and wastewater discharge outlets.

Figure 2. A schematic of the automated system for the simultaneous decontamination of 10 washbasin U-bends, drain outlets and wastewater pipes by sequential treatment with catholyte followed by anolyte used in the present study. Only four washbasins are shown for clarity. Each U-bend had two ports to facilitate sampling. The lower part of the figure shows a process control schematic for automated decontamination. The programmable process controller initiates treatment cycles. At the start of each cycle the process controller sends a signal to the actuator to close the valve on the wastewater outflow pipe. After a 30 s delay, a signal activates the catholyte dosing pump for 3.5 min and catholyte is pumped into the pipework below the washbasin U-bends until the pipework and U-bends are completely filled to a level a 5 cm above the washbasin drain outlets. Catholyte is left *in situ* for 10 min, after which time the process controller opens the valve voiding catholyte to the wastewater stream. The valve is then closed and after a 30 s delay the process controller activates the anolyte dosing pump for 3.5 min and the cycle proceeds as per catholyte dosing. After 10 min the anolyte is voided to waste completing the cycle.

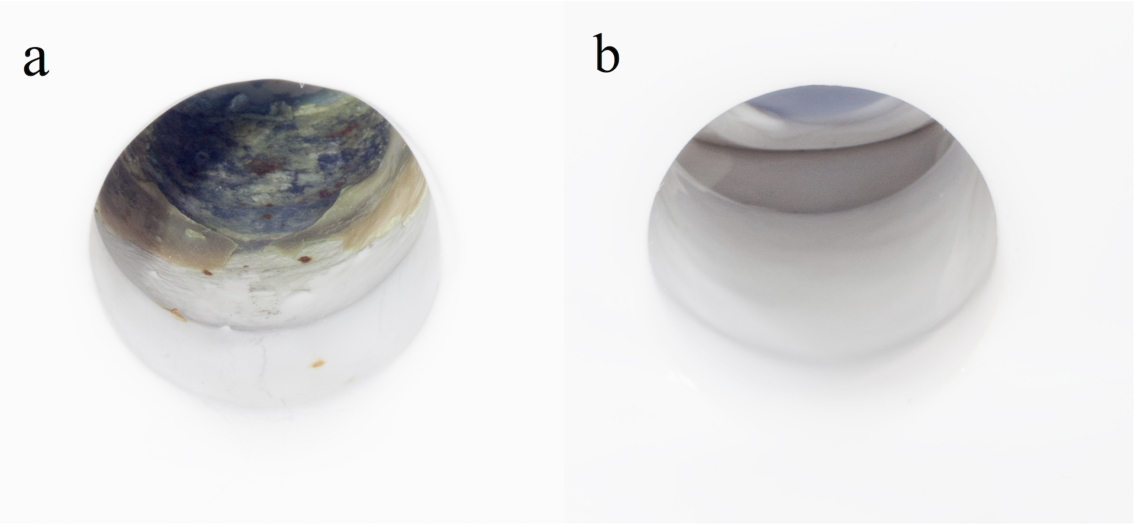
Supplemental Table I. Bacterial species recovered from U-bends during the study

Bacterial species identified in ECA-treated U-bends	Bacterial species identified in non ECA-treated U-bends
Gram-positive	
Aerococcus viridans	*Brevibacterium casei*
Bacillus cereus	*Micrococcus luteus*
Bacillus pumilus	
Bacillus simplex	
Micrococcus luteus	
Staphylococcus aureus	
Staphylococcus capitis	
Staphylococcus cohnii	
Staphylococcus epidermidis	
Staphylococcus hominis	
Staphylococcus saprophyticus	
Staphylococcus warneri	
Gram-negative	
Acinetobacter ursingii	*Aeromonas hydrophila*
Acinetobacter johnsonii	*Acinetobacter junii*
Acinetobacter radioresistens	*Acinetobacter ursingii*
Aeromonas hydrophila	*Citrobacter freundii*
Brevundimonas diminuta	*Cupriavidus pauculus*
Chryseobacterium indologenes	*Delftia acidovorans*
Citrobacter freundii	*Enterobacter hormaechei*
Cupriavidus pauculus	*Hafnia alvei*
Delftia acidovorans	*Pseudomonas aeruginosa*
Enterobacter cloacae	*Pseudomonas fluorescens*
Hafnia alvei	*Pseudomonas putida*
Klebsiella oxytoca	*Raoultella ornithinolytica*
Raoultella ornithinolytica	*Rhizobium radiobacter*
Stenotrophomonas maltophilia	*Stenotrophomonas maltophilia*
Pseudomonas aeruginosa	
Pseudomonas fluorescens	
Pseudomonas putida	

Supplemental Figure S1



Supplemental Figure 2



Supplemental Figure legends

Supplemental Figure S1.

Electron miscroscope images of sections of the internal surfaces of an ECA-treated U-bend (upper panel) and and untreated U-bend (lower panel). The ECA treated section is totally free of biofilm, whereas the untreated section harbours dense biofilm. Both sections were taken from the U-bends shown in Figure 1b from the areas immediately above the waterline of sampling surface 1.

Supplemental Figure S2

Photographs of (a) a control and (b) an ECA-treated washbasin drain outlet at the end of the study. The U-bend and drain outlet of the treated washbasin were subjected to 62 cycles of ECA treatement over five months. The treated drain outlet is noticibly free from visible biofilm, whereas the control drain outlet contains visible biofilm.