Minimisation of Microbial Reservoirs and Infection Risks From Washbasin U-bend Traps Using a Novel Automated Disinfection System with Electrochemically Activated Solutions

A thesis submitted to the University of Dublin in fulfilment of the requirements for the degree of Master in Science

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Declaration

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James S. Swan

“If opportunity doesn’t knock, build a door”

Milton Berle

Summary

Background: Washbasin and sink U-bends are ubiquitous in virtually all buildings including hospitals and smaller healthcare premises. They have been used for over a century and their principal purpose is to prevent foul odours emanating from wastewater pipes from entering buildings. U-bends are designed to retain a small volume of water, which acts as the seal preventing the passage of sewer gas from wastewater pipework. The water retained in U-bends is frequently stagnant, which encourages the growth and proliferation of microbial biofilms, populated predominantly by Gram-negative bacterial species and especially by *Pseudomonas aeruginosa*. Over the past two decades there have been numerous reports of nosocomial outbreaks of infection related directly or indirectly to contaminated U-bends. Three different approaches at addressing this problem have been used previously including the application of chemical disinfectants such as bleach, the replacement of sanitary fixtures and/or U-bends and the use of a U-bend heating element together with vibrational cleaning. The first two approaches worked well in several studies reducing contamination and infection risks in the short term, but failed to provide a long-term solution due to recolonisation of U-bends and the associated pipework with biofilm. The use of U-bend heating elements together with vibrational cleaning was shown to be effective, but the approach is expensive as the heating elements are in constant operation.

Electrochemically activated (ECA) solution generators produce two solutions through activation of dilute brine. These include a metastable oxidant solution termed ‘anolyte’ (predominantly hypochlorous acid (HOCl)) and a second solution termed ‘catholyte’ with detergent properties (predominantly sodium hydroxide (NaOH)) The solutions are generated by passing a brine solution through a flow-through electrolytic cell. Anolyte is highly microbiocidal and capable of penetrating biofilms. Previous studies from this laboratory have shown that treatment of water supplied to dental units and washbasins with residual anolyte consistently eliminates biofilm and provides output water virtually free of contamination.

Aim: The purpose of this study was to investigate whether periodic treatment of washbasin U-bends and associated pipework with catholyte solution as a cleaning agent followed by anolyte solution as a disinfectant could minimise biofilm contamination of U-bends. Achieving this objective would require developing an approach to seal the wastewater outflow pipework from washbasins so that U-bends and associated pipework could be

completely filled with ECA solutions for specified periods of time for maximum efficacy. A second objective was to automate U-bend decontamination with ECA solutions.

Methods: Initially three identical washbasin U-bends were manually filled with catholyte solution followed by anolyte solution for five min each once weekly for five weeks. Three additional identical washbasin U-bends were used as controls. A programmable system was then developed with one washbasin that automated this process using an actuator-controlled ball valve to seal the wastewater outlet pipe and chemical-resistant dosing pumps to dose ECA solutions into the U-bend and associated pipework. A Programmable Logic Controller was used to coordinate the sequence of operation of the actuator and dosing pumps. 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 following each round of ECA treatment on blood agar (CBA), R2A, PAS and PA agars following automated treatment and on CBA and R2A following manual treatment. Bacterial identification was determined by comparing small ribosomal subunit rRNA gene sequences with consensus sequences for individual bacterial species in the EMBL/GenBank databases.

Results: The average bacterial density from untreated U-bends throughout the study was >1 x 10^5 CFU/swab on all media with *Pseudomonas aeruginosa* accounting for approximately 50% of bacterial counts. Manual treatment of U-bends with ECAs reduced counts significantly (<100 CFU/swab) (P <0.01 for CBA; P <0.005 for R2A). *Pseudomonas aeruginosa* was eliminated from the U-bend subjected to automated ECA-treatment, with average bacterial 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. Following the three-month study period, the ECA-treated and control U-bends were removed and cut in cross-section and segments examined by electron microscopy, which revealed the virtual elimination of biofilm from the ECA-treated U-bend. In contrast, the control U-bend was heavily fouled with dense pigmented biofilm.

Conclusion: Microbial contamination of washbasin U-bends can be consistently minimised by automated decontamination with ECA solutions.

Future Developments: Work is in progress to develop a large-scale system for simultaneous automated decontamination of multiple washbasin U-bends.

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life are an inspiration to me. To Conor and Liam, the best has yet to come. To my mother, sadly missed, I hope I've made you proud.

Abbreviations

A&E	Accident & Emergency
BMS	Building Management System
BSA	Bovine serum albumin
BSP	British Standard Pipe
CAT 5	Category 5
CBA	Columbia Blood Agar
CFU	Colony forming units
CIP	Clean In Place
cm	Centimetre
CPEs	Carbapenemase-producing *Enterobacteriaceae*
˚C	Degrees Centigrade
DDUH	Dublin Dental University Hospital
DNA	Deoxyribonucleic acid
DUWLs	Dental units waterlines
ECA	Electrochemical activation
EN	European Norm (relates to European Standards)
ESBLEs	Extended spectrum β-lactamase producing *Enterobacteriaceae*
et al.	and others
FAC	Free available chlorine
FEM	Flow-through electrolytic module
FPM	Fluoro-rubber
h	Hour
HBN	Health Building Note

HFN	Health Facilities Note
HMI	Human Machine Interface
HOCL	Hypochlorous acid
HPSC	Health Protection Surveillance Centre, Ireland
HTM	Health Technical Manual
ICU	Intensive care unit
in-situ	Left in position
IPC	Infection prevention and control
IPS	Integrated plumbing system
KDF	Kinetic degradation fluxion
LAN	Local area network
L	Litre
Ltd.	Limited
m	Meter
ml	Millilitre
mm	millimetre
mV	Millivolts
NaOH	Sodium hydroxide
ppm	Parts per million
PE	Polyethylene
PVC	Polyvinyl chloride
RHE	Reconstituted human oral epithelium
rRNA gene	Small ribosomal subunit rRNA gene
PA	*Pseudomonas* selective agar

PAS	*Pseudomonas aeruginosa* selective agar
PLC	Programmable logic controller
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride
R2A	Reasoner's agar
s	Second
TE	Tris ethylenediaminetetraacetic acid
USA	United States of America
UK	United Kingdom
WRSA	Water Regulations Regulatory Scheme
w/v	weight by volume
v/v	volume by volume
µm	micrometre
µl	microliter
%	percentage
≤	less than or equal to
≥	greater than or equal to

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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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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 5 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 5 min catholyte followed by 5 min anolyte treatment per 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 $>1\times10^5$ cfu/swab on all media with *Pseudomonas aeruginosa* accounting for ~50% of counts. Manual U-bend electrochemically activated (ECA) solution 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 for 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 ($P<0.05$) cfu/swab, respectively. *P. aeruginosa* was eliminated from all treated U-bends.

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

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Introduction

Hospital water systems and associated fixtures and fittings have been identified as significant reservoirs of microbial contamination responsible for nosocomial infections, especially 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 may 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 aerosolization facilitate retrocontamination 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 colonized with micro-organisms.[2] Disinfectants have diminished efficacy against dense biofilms present in U-bends and associated pipework, and, whereas they may 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 minimize 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 minimizing microbial contamination.

Methods

Chemicals

All chemicals and reagents used were of analytical or molecular biology grade and were purchased from Sigma–Aldrich (Arklow, 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, FL, 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

Free available chlorine levels in anolyte were measured using a Hach Pocket Colorimeter II (Hach Company, Ames, IA, USA) according to the manufacturer's instructions.

Test and control washbasins

Six identical ceramic washbasins (Armitage Shanks, Rugeley, UK) located in adjacent staff bathrooms at the Dublin Dental University Hospital were included in the pilot study. All bathrooms are in frequent use Monday to Friday. Three months prior to the study, washbasins were equipped with new Multikwik polypropylene U-bends (Marley Plumbing and Drainage, Maidstone, UK) 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 minimize 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 (~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 5 min and the valve was then 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 was swab-sampled through the cleaning

ports using swabs soaked in neutralization 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, was fitted to the wastewater pipework downstream from the U-bend replacing the manual valve used in preliminary experiments. The actuator and pumps were 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 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-filled the U-bend and caused air and water from the U-bend to rise into the washbasin through the wastewater outlet opening. Catholyte was left *in situ* for 5 min and then voided to waste by automated opening of the valve. Following a 20 s delay, the actuator closed the valve and following a further 20 s delay a second pump dosed anolyte into the system and the cycle proceeded 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, Brescia, 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 neutralize 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 1 min, serially diluted, and 100 μL aliquots spread in duplicate on to Columbia blood agar (CBA)

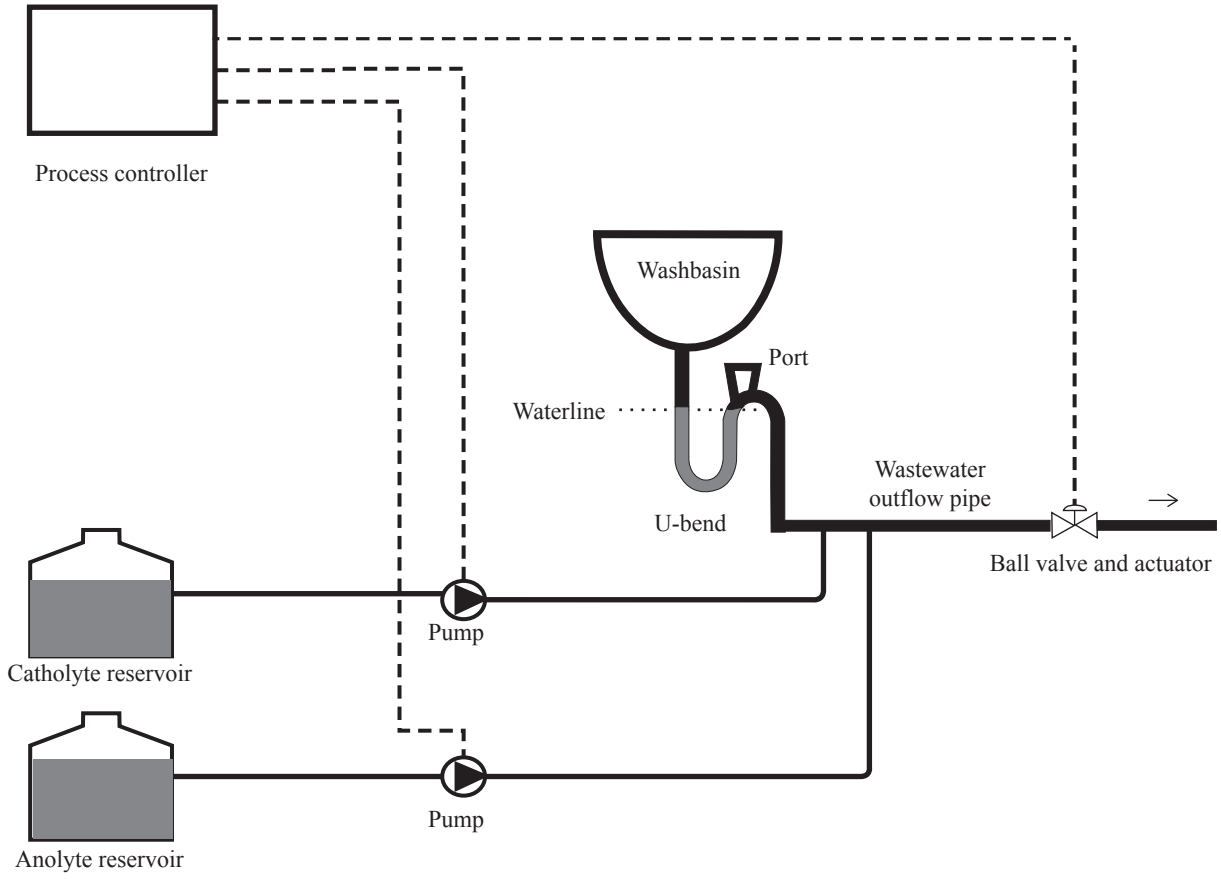

Figure 1. Schematic diagram of automated washbasin U-bend treatment. Treatment cycles are initiated by the programmable process controller. At the start of each cycle the actuator closes the valve on the wastewater outflow pipe. After a 20 s delay, catholyte is pumped into the pipework below the washbasin U-bend until the pipework and U-bend are completely filled to a level a few centimeters 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 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.

(Lip Diagnostic Services, Galway, Ireland), R2A agar (Lip), *P. aeruginosa* Selective Agar (PAS) (Oxoid Ltd, Basingstoke, UK) 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 that 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 (cfu) 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, Bromborough, UK) 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, CA, USA). Statistical significance was determined using an unpaired, two-tailed Student's *t*-test with 95% confidence interval (CI).

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 that all were heavily contaminated. The mean average bacterial density on CBA and R2A agars was 2.41×10^5 ($\pm2.5\times10^5$) and 1×10^6 ($\pm9.9\times10^5$) cfu/swab, respectively (CBA range 4.8×10^3 to 7.6×10^5 cfu/swab; R2A range 9.2×10^3 to 3.8×10^6 cfu/swab). By 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 5 min followed by anolyte for a further 5 min for a three-month period (35 cycles in total). Neutralized 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 1×10^5 cfu/swab in each case (Table I). By 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 cfu/swab, respectively (Table I). For all four media the 5-$\log_{10}$ reduction in bacterial density achieved between the ECA-treated and untreated U-bends was significant (Table I). 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 I). Culture analysis of neutralized 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*. *P. aeruginosa* accounted for ~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 minimize microbial contamination in washbasin U-bends using regular automated treatment. Because water stagnation in U-bends may result in especially 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

Table I
Comparative bacterial counts from a washbasin U-bend subjected to automated treatment with electrochemically activated solutions and an untreated U-bend during a three-month period

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 to 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 to 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 to 7.80×10^5	
PAS	Treated	0	0	0	<0.05
	Untreated	1.02×10^5	2.49×10^5	2×10^3 to 1.3×10^6	
		Counts[b] 24 h after treatment ($N = 18$)			
CBA	Treated	35.28	83.48	0–350	<0.001
	Untreated	1.18×10^5	1.24×10^5	9.5×10^3 to 5×10^5	
R2A	Treated	82.22	199.4	0–845	<0.01
	Untreated	1.76×10^5	2.46×10^5	7×10^3 to 1×10^6	
PA	Treated	16.11	39.95	0–155	<0.01
	Untreated	5.9×10^4	6.82×10^4	1×10^3 to 2×10^5	
PAS	Treated	13.89	33.81	0–125	<0.01
	Untreated	3.84×10^4	5.56×10^4	1×10^3 to 2×10^5	

cfu, colony-forming units; CBA, Columbia blood agar; R2A, R2A agar; PA, *Pseudomonas* selective agar; PAS, *P. aeruginosa* selective agar; SD, standard deviation.

[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 neutralized swabs. In 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.

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 and <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 $>1\times10^5$ cfu/swab (Table I), whereas microbial contamination of the ECA-treated U-bend was virtually eliminated (Table I). Furthermore, sampling of U-bends 24 h after treatment showed minimal contamination relative to controls (Table I). 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 *et al.* 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 *et al.* using bleach to control β-lactamase-producing-Enterobacteriaceae in sink wastewater outlets found that daily disinfection was significantly more effective than weekly.[4] A recent laboratory study suggested that the use of copper pipework in sink wastewater outlets may exhibit higher antimicrobial activity than widely used polyvinylchloride pipework.[12] However, it is unknown whether the antimicrobial effect of copper would be sustained in the long term, as copper may develop oxidation layers over time.

Pseudomonas aeruginosa was the most prevalent and abundant bacterial species present in untreated U-bend samples, accounting for ~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 minimizing microbial contamination of dental unit waterlines and washbasin output water in the long term (more than two 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 nor anolyte alone are effective at minimizing 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 found that self-

disinfecting U-bends with a heating element to heat U-bend wastewater to ≥85°C followed by vibration cleaning were 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 ~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 that 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 minimize microbial contamination of water networks and taps, so no additional costs relating to the purchase of ECA solutions were incurred.[9—11] The additional one-off costs for automated U-bend treatment for up to 10 washbasin U-bends would be about €5,000, with annual running costs of about €200 and staff time requirement of about 20 min per week.

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

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Conflict of interest statement
None declared.

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Chapter 1 Introduction

1.1 Introduction

The role of the healthcare environment in hospital-acquired infections is well recognised (Dancer *et al*., 2014). Healthcare water systems have increasingly been identified as significant reservoirs of microbial contamination responsible for nosocomial infections, particularly among immunocompromised, neonatal and high-dependency patients in areas such as intensive care units (ICUs) (Cholley *et al*., 2008; Hota *et al*., 2009; La Forgia *et al*., 2010; Decker and, Palmore, 2013; Loveday *et al*., 2014; Walker *et al*., 2014; Blom, 2015; Bloomfield *et al*., 2015; Capelletti and Moraes, 2016). Microbial biofilms form readily within washbasin and sink wastewater outlets/drains and associated pipework because these areas are frequently moist or wet (Cholly *et al*., 2008; Hota *et al*., 2009; La Forgia *et al*., 2010; Breathnach *et al*., 2012; Bloomfield *et al*., 2015; Capelletti and Moraes, 2016). This includes the water retaining U-bend, also known as a trap, that prevents sewer gas entry into buildings via wastewater pipes. However, U-bends also collect hair, skin cells and other debris, encouraging the growth of biofilms (Coleman *et al*., 2010). These biofilms can contain a range of opportunistic bacterial pathogens including *Pseudomonas*, *Stenotrophomonas*, *Serratia*, *Burkholderia*, *Acinetobacter*, *Klebsiella*, *Citrobacter* and non-tuberculosis mycobacteria spp. amongst others, many of which can be resistant to the major classes of antibiotics (Perryman and Flournoy, 1980; Denton *et al*., 1998; Donlan, 2000; Pitmen *et al*., 2001; Denton *et al*., 2003; Hota *et al*., 2009; La Forgia *et al*., 2010; Bloomfield *et al*., 2012; Breathnach *et al*., 2012; Lowe *et al*., 2012; Starlander and Melhus, 2012; Decker and Palmore, 2013; Roux *et al*., 2013; Vergara-López *et al*., 2013; Wolf *et al*., 2014; Blom, 2015; Leitner *et al*., 2015; Wendel *et al*., 2015; Chapuis *et al*., 2016; Tissot *et al*., 2016). Of particular importance are reports highlighting the role of hospital washbasins drains as a source of nosocomial transmission of carbapenemase-producing *Enterobacteriaceae* (Denton *et al*., 1998; Denton *et al*., 2003; Vergara-López *et al*., 2013; Leitner *et al*., 2015; Soothill, 2016; White *et al*., 2016; DeGeyter *et al*., 2017).

Carbapenemase-producing *Enterobacteriaceae* have emerged relatively recently as a major health threat in hospitals and the community and only a few antimicrobial agents remain active against these microorganisms (Doi *et al*., 2015; Meletis, 2016). Protozoa including *Acanthamoeba* spp. and *Nagelleria fowleri* are also of concern (Wang *et al*., 2017). The mixed biofilm communities present in U-bends can not only harbour antimicrobial resistance elements but can exchange them with susceptible strains making infections caused by these organisms difficult to treat (Donlan, 2002; Hota *et al*., 2009; La Forgia *et al*., 2010: Breathnach *et al*., 2012; Muzslay *et al*., 2017). Conjugative and mobilisable plasmids and transposons are among the mobile genetic elements frequently present in these species that commonly harbour antimicrobial agent resistance genes (Donlon, 2002). The motility of some of these species, especially *Pseudomonas aeruginosa*, which together with water flow, splashing and aerosolisation facilitate retro-contamination of washbasins, sinks and taps from the hospital ward environment, often with serious consequences to patients (Döring *et al*., 1991; Hota *et al*., 2009; Breathnach *et al*., 2012; Decker and Palmore, 2013; Loveday *et al*., 2014; Fusch *et al*., 2015; Salm *et al*., 2016). Anaissie *et al*. (2002) estimated that waterborne *P. aeruginosa* nosocomial pneumonias kills over 1400 patients per annum in the USA, and argued that the total number of deaths due to nosocomial waterborne pathogens may dramatically exceed this figure. Worldwide reports detail an increasing number of outbreaks in hospitals due to multidrug-resistant Gram-negative bacteria associated directly or indirectly with contaminated washbasin and sink drains and U-bends (Pitmen *et al*., 2001; Hota *et al*., 2009; La Forgia *et al*., 2010; Breathnach *et al*., 2012; Lowe *et al*., 2012; Starlander and Melhus, 2012; Roux *et al*., 2013; Vergara-López *et al*., 2013; Wolf *et al*., 2014; Leitner *et al*., 2015; Wendel *et al*., 2015; Chapuis *et al*., 2016; Salm *et al*., 2016; Amoureux *et al*., 2017; De Geyter *et al*., 2017).

Water systems in hospitals have been identified as significant reservoirs of microbial contamination responsible for nosocomial infections. Hospital water delivery networks, wastewater networks, low water flow rates, inadequate water temperature controls, taps, sinks and washbasins can provide ideal conditions for microbial proliferation, especially if poorly maintained or installed incorrectly. Water in use at washbasins in a clinical setting provide ideal conditions for bacterial growth. Water tanks, pipes, washbasins, showers, U-bends and all associated fixtures and fittings provide reservoirs for water stagnation and ultimately sources of bacteria (Dancer, 2014; Walker and Moore, 2015). As mentioned in the preceding paragraph, numerous nosocomial infections and outbreaks caused by multidrug-resistant bacteria, especially Gram-negative species, have been attributed to these. These infections are particularly onerous in a healthcare environment among vulnerable patient groups, such as the elderly, the immunocompromised and patients with other underlying medical conditions. The risk of infection to vulnerable patients makes prevention of waterborne infections in modern healthcare facilities a high priority. Several outbreaks, including recent incidents involving neonates in Northern Ireland in 2011/2012, have been attributed to contaminated water systems (Walker *et al*., 2014). As a direct result of the deaths of four neonates in Northern Ireland, guidance documents and addendums to Health Technical Memorandums 01-04 (Department of Health, UK, 2016b) were produced to advise National Health Service managers on how to deal with the presence of *P. aeruginosa* in healthcare units (Walker and Moore, 2015). These guidance documents detail procedures for routine water testing at six-monthly intervals with directed interventions such as disinfection and replacement of high-risk plumbing components. Studies have shown that conventional methods of disinfection of washbasins and U-bends are ineffective in stemming or eliminating the growth of biofilm. Microbial biofilms readily form in areas where water is used, particularly at sinks and washbasins, U-bends, drains and all associated pipework (Walker *et al*., 2014). U-bends are particularly prone to

biofilm growth, as they are designed to retain water that is frequently stagnant. Management of water systems to reduce the risk of microbial growth including opportunistic pathogens such as *Legionella* and *P. aeruginosa* is vital to patient safety. It requires surveillance and maintenance of control measures including temperature control, usage, cleaning and disinfection measures as identified within the risk assessment and *Legionella* control scheme for both hot- and cold-water systems (Health Protection Surveillance Centre, Ireland, 2015).

1.2 Hand washbasins in the healthcare environment

Biofilms can develop in hand washbasins, associated splashbacks, taps and U-bends and all can act as reservoirs and disseminators of infection (Coleman *et al*., 2010). These sources of biofilms are common place in healthcare settings and patient exposure to the microorganisms present in biofilms can occur with these fixtures. Any personal contact with these fixtures or indirect contact via items of equipment contaminated with microorganisms from these fixtures can result in transmission of microorganisms to patients with the potential for subsequent infections. Contaminating microorganisms may originate from biofilms, sediment in water tanks or other water distribution components.

Irish national guidelines for the prevention and control of infection from water systems in healthcare facilities issued by the Health Protection Surveillance Centre (HPSC) in 2015 (Health Protection Surveillance Centre, Ireland, 2015) refer specifically to the required design of sanitary assemblies within a healthcare premises. These guidelines refer to the UK Department of Health, Health Building Note (HBN) 00-10 Part C-Sanitary Assemblies (Department of Health, UK, 2013), which provides detailed requirements for clinical hand washbasins. The main purpose of this HBN is to significantly reduce the risk of contamination and the spread of microorganisms from sanitary assemblies. In practice, this means reducing or eliminating surface areas with nooks or crannies that can retain bacteria. It also means closely monitoring the water supply to mitigate the risk of

contamination. This HBN provides information on waste disposal units, waste pipework, urinals, showers, basins and baths, to name but a few. It also covers the relationships between the appliance, fittings and pipework within the clinical environment. All sanitary equipment used in a healthcare setting, at a minimum, should comply with the Water Regulations Advisory Scheme (WRAS, UK). The HBN memorandum details two categories of washbasins known as general pattern (Figure 1.1) and hospital pattern (Figure 1.2) assemblies. Specifications for washbasin layout, dimensions and taps etc. for use in the healthcare environment are provided in Health Facilities Note 30 (HFN 30) (Department of Health, UK, 2002), which has recently been superseded by Health Building Note (HBN) 00-09 (Department of Health, 2013). These documents highlight the major infection prevention and control (IPC) issues in the built environment and the risks to address to minimise infection risks.

Vitreous china washbasins are widely used in clinical settings and are regarded as an important element in the design of patient treatment areas. This ensures a smooth, easy to clean washbasin surface with no areas prone to bacterial accumulation. Building service engineers are generally tasked with the responsibility of providing appropriate and adequate clinical facilities including sanitary fittings and appliances, which must comply with European Standards EN 1111:1999 (Anonymous, 1999) and EN 274-2:2002 (Anonymous, 2002). Initially hand washbasins were installed in healthcare premises to reduce the risk of transmission of microorganisms and to reduce the spread of infection. Handwashing in the healthcare setting has been promoted for many years and is recognised as the single most important procedure for preventing infection (World Health Organization, 2009). In the 1970's guidelines were published for the clinical environment advocating handwashing with soaps without antimicrobial agents, while retaining the use of antimicrobial soaps for use before and after invasive procedures (Health Protection Surveillance Centre, Ireland, 2009). Engineers involved in the design of hospital

Figure 1.1 Photograph showing a typical general pattern washbasin. The washbasin has an overflow outlet in the upper back wall of the washbasin and the tap water flow impacts the drain outlet directly. Water impacting the drain outlet can readily aerosolise bacteria from the U-bend beneath the washbasin. This type of washbasin is typically used in a domestic setting but should not be used in a healthcare facility and especially not in critical care areas such as intensive care units and hospital areas with vulnerable patients.

Figure 1.2 Photograph showing a typical hospital pattern washbasin suitable for use in a clinical setting. The washbasin does not have an overflow outlet and the wastewater drain is offset in the back wall of the washbasin to minimise aerosolisation of bacteria from the drain outlet and associated pipework when tap water is flowing.

infrastructure increased the number of hand washbasins in clinical facilities for the purposes of promoting good hand hygiene. Paradoxically, these practices while facilitating more frequent hand washing resulted in increased associated microbiological risks.

All hand washbasins must have a U-bend installed on the wastewater outlet, and it is these that have largely contributed to infection risks associated with the use of hand washbasins. The design of sinks, hand washbasins and associated plumbing used in the clinical setting has changed over the years, and while washbasins and taps can vary in size, the principles of their design and operation remain the same. A Washbasin should only allow handwashing under a stream of running water; mixer taps allow this to be practised safely in healthcare settings where hot water temperatures may be high to control the proliferation of *Legionella* bacteria (Health Technical Memorandum 04-01, Part A) (Department of Health, UK, 2016a). *Legionellae* are environmental bacterial species found naturally in water and soil where they exist predominantly as intracellular parasites of a variety of protozoa and amoebae. They grow best between 25-45°C, do not grow or grow very slowly <20°C and are killed at >60°C. *Legionella*e can thrive in building water systems that are poorly designed and/or maintained and especially when water stagnation occurs (Health Protection Surveillance Centre, Ireland, 2009). There are a wide variety of control measures, which if applied correctly and consistently, can significantly reduce legionellosis risks in buildings. The most basic control measure involves maintaining cold water supplies <20°C, producing hot water at >60°C and circulating hot water at >50°C. Hot water returning to calorifiers (i.e. boilers) should be approximately 50°C. Thermal controls can effectively minimise *Legionella* multiplication, however the application of these controls introduces opportunities for scalding during the use of hand washbasins. It is therefore imperative that exposure to hot water above 40°C in the clinical environment must be avoided which is usually achieved through the use of thermostatic controls within the tap housing (Health Protection Surveillance Centre, Ireland, 2015).

The taps on a hospital pattern washbasin should not be integrated into the surface of the washbasin itself (Figure 1.2). Taps should be wall mounted on an Integrated Plumbing System (IPS) panel, behind which all the plumbing services are concealed. Balancing the hot water system flow and return circuits is critical to avoid long lengths of stagnant pipework that are likely to be at a lower temperature. Where the hot water system return is local to the washbasin outlet a much quicker response should be achieved (i.e. <30 seconds, typically <10–20 s).

The washbasin wastewater outlet or drain should be offset in the back wall of the washbasin without a plug and should be free draining. The dimensions of a clinical hand washbasin should be large enough to contain most splashes and therefore enable effective hand washing without excessive splashing. The washbasin splash back should also be sealed and waterproof to allow effective cleaning of all surfaces. Variations in suitable taps range from automated low voltage taps with temperature control to spring loaded knee operated systems (Health Protection Surveillance Centre, Ireland, 2015).

The use of suitable hospital pattern clinical washbasins is not universally implemented. Regardless of appropriate washbasin design, biofilms still form within the wastewater outlet and pipework connected to it. Hospital sinks used for purposes other than hand washing frequently have overflows and drains that are impacted directly by tap water flow. These too can act as reservoirs of contamination seeding the hospital environment. Shower wastewater outlets called traps carry out a similar function to U-bends on sink outlets and have been implicated in nosocomial infections and outbreaks of *P. aeruginosa* (Breathnach *et al*., 2012; Quick *et al*., 2014; Blom, 2015; Tissot *et al*., 2016). A hand washbasin in a clinical setting must minimise the production of contaminated aerosols when the taps are being used. The drain outlet of the washbasin must be offset from the inlet spout of the taps such that the inlet water stream does not flow directly into the wastewater drain outlet, reducing the risk of splashing and aerosolisation

of bacteria (Department of Health, UK, 2013). Clinical washbasins must not have a plug or overflow outlet directly under the inlet stream to avoid dislodging the biofilm and aerosolisation of bacteria.

1.3 Clinical washbasin taps

There are a variety of suitable taps available for use in a clinical setting including hand/elbow operated taps, long lever bib taps, long lever high neck pillar taps, single lever wall mounted (bib) mixer tap and short lever operated basin taps (Department of Health, UK, 2013). The use of swan neck taps should be avoided on a clinical wash hand basin as such taps do not empty fully after use and thus provides a potential dead leg within the tap (Department of Health, UK, 2013)

Health Protection Surveillance Centre guidelines for the prevention and control of infection from water systems in healthcare facilities should be consulted when considering the problems of safety of water in a healthcare environment (Health Protection Surveillance Centre, Ireland, 2015). The safety of users, particularly children and older people, would be compromised if they were allowed to use washing facilities supplied with water at circulating hot water temperature. This risk can be reduced by the installation at each hot water outlet of a locally adjustable thermostatic mixing valve. A thermostatic valve mixes (set automatically or manually) the hot and cold waters supplies within the tap producing water temperature between 35°C and 40°C. Valves of this type are unaffected by changes in water pressure and should automatically and quickly close the hot or cold supply if either fails.

Sensor operated taps are a particularly suitable option for use with washbasins in a clinical setting and provide a non-touch solution thus reducing contamination risks (Department of Health, UK, 2013). They also avoid undue use of valuable water resources. The sensor operates the taps when the operator puts their hands in the sensor detection zone and shuts off automatically after a preset time. The sensor range is adjustable for

operator suitability. The sensor opens the thermostatic valve providing water between 35°C and 40°C for hand washing. These electronic taps also offer solutions for routine pre-timed flushing via a Building Management System (BMS) if the taps are not in regular use, thus minimising issues of microbial proliferation associated with water stagnation. A flushing kit is available for modern taps used in a clinical setting. This kit provides a facility for carrying out thermal disinfection of both the hot and cold taps as well as the thermostatic body (Department of Health, UK, 2016). Most manufacturers can supply demountable taps where are designed to be easily removed for maintenance purposes. Where such taps are in use they should be periodically removed for descaling and decontamination followed by sterilisation by autoclaving.

Tap aerators or flow straighteners are devices inserted into the spout outlet of a tap to modify flow, reduce turbulence and create an even stream of water (Department of Health, UK, 2016). They restrict the flow of water from taps without reducing water pressure. Aerators fitted to taps can reduce the amount of water used at the washbasin by more than 49% (Meireles *et al*., 2017). The aerator consists of a housing, insert and washer, which can be dismantled and disinfected to reduce contamination risks (Figure 1.3). Taps fitted with aerators introduce air into the water stream. This alters the stream and reduces water splash converting the stream from turbulent to laminar flow thus reducing the aerosolisation of waterborne bacteria and bacteria present in biofilms in taps. Ideally new installations of clinical pattern washbasins taps should not employ an aerator as they introduce a requirement for additional routine maintenance (including decontamination) to reduce the risk of bacterial contamination. However, in reality most hospitals are equipped with a variety of washbasins and taps, including hospital pattern and non-hospital pattern washbasins and taps with and without aerators. There are a variety of reasons for this including the lack of adequate funding, lack of knowledge, additions and alterations to healthcare facilities over time and change of function of individual areas over time.

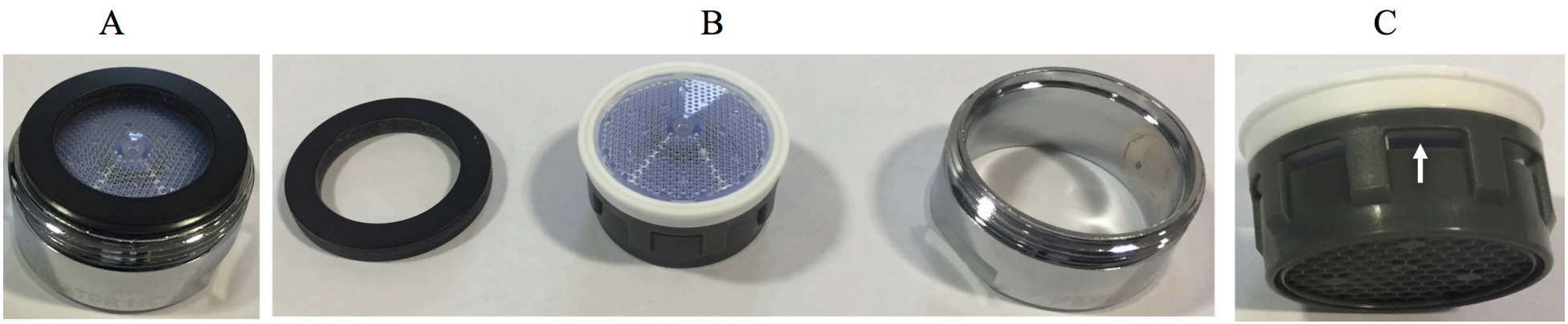

Figure 1.3 Photographs showing an example of tap aerator and its components. Aerators consist of a housing, aerator insert and washer, which can be dismantled and disinfected to reduce contamination risks. Taps fitted with aerators introduce air into the water stream, which alters the stream and reduces water splash converting the stream from turbulent to laminar flow thus reducing the aerosolisation of waterborne bacteria and bacteria present in taps and washbasins. Panel A shows an assembled aerator. Panel B shows the aerator shown in panel A following disassembly showing the washer (left), the aerator unit (centre) and the housing (right), the latter of which attaches directly to a tap. Panel C shows a side view of the aerator unit with one of the air inlets indicated by a white arrow.

1.4 U-bends

A U-bend is a building services component specified by engineers at the design stage of the building structure. A U-bend will be specified in the mechanical design criteria where hand washbasins and sinks are employed. The size and configuration will be designed for the size and volume of the washbasin/sink as well as the flow rate (hot and cold water) into the washbasin/sink. The dimensions of the outflow wastewater pipe are configured so that the washbasin or sink will not overflow while the hot and cold water supplies are running; the outlet volume flow is greater than the inlet flow. Biofilm contamination of sinks/washbasins can largely be attributed to the U-bends as these retain a volume of water within the lower portion of the bend, which can remain stagnant if the fixture is not in frequent use. The U-bend is designed to create a water seal between the washbasin/sink fixture and foul gases that are generated in wastewater pipes. The water seal prevents foul air escaping from the drain back up into the building (Figure 1.4). The U-bend can also act as a trap for heavy items entering the drain.

There are wide varieties of U-bends, sometimes called bottle traps or P-traps etc., available and these will be specified for the intended function of a sink/washbasin as well as the flow rates (Environment, Heritage and Local Government. Building Regulations, 2010). In all cases, the primary function of the U-bend is to stop foul gas emissions escaping back up through the pipework. By design, therefore the U-bend will constantly retain a volume of water, which can be stagnant for sustained periods of time and thus encouraging the proliferation of microbial biofilm (Figure 1.5).

These microbial biofilms coat the moist areas of pipework and therefore build up easily within the U-bends. The biofilm also builds up in the pipework directly above the retained water level as far as the sink drain outlet and also below the U-bend (Kotay *et al*., 2017). In the healthcare environment, U-bends can act as disseminators of infection by the range of bacteria present within the biofilm. The density of bacteria present in drains beneath

washbasins in hospitals has been estimated to range from 10^6 -10^{10} colony forming units per ml of which 10^3-10^5 CFU/ml are Gram-negative bacteria, particularly waterborne bacterial species (Döring *et al*., 1991). Contaminated sinks and washbasins have been implicated directly or indirectly in numerous nosocomial outbreaks (Cholly *et al*., 2008; Hota *et al*., 2009; Breathnach *et al*., 2012; Lowe *et al*., 2012; Starlander and Melheus, 2012; Decker and Palmore, 2013: Roux *et al*., 2013; Vergara-López *et al*., 2013; Blom, 2015; Leitner *et al*., 2015; Chapuis *et al*., 2016; Salm *et al*., 2016; Herruzo *et al*., 2017; Varin *et al*., 2017). Biofilm present in washbasin pipework is difficult to eradicate by disinfection alone and often the bacteria present in these biofilms are motile, especially *Pseudomonas aeruginosa* and other Gram-negative species, allowing them to retro-contaminate washbasins, sinks and taps (Hota *et al*., 2009; Loveday *et al*., 2014; Salm *et al*., 2016; Amoureux *et al*., 2017; Kotay *et al*., 2017).

1.5 Previous approaches to decontaminating washbasin and sink drains

Eradicating biofilms that occur in sinks and U-bends is difficult. Approaches that have been previously considered have involved replacing the sinks and plumbing fixtures including U-bends, using high temperature water within the U-bends and using acetic acid, bleach and other chemicals (Hota *et al*., 2009; La Forgia *et al*., 2010; Lowe *et al*., 2012; Vergara-López *et al*., 2013; Wolf *et al*., 2014; Fusch *et al*., 2015; Leitner *et al*., 2015; Wendel *et al*., 2015; Stjärne Aspelund *et al*., 2016). These approaches have largely proved ineffective, are cost prohibitive or present other safety concerns.

Difficulties in eradicating biofilms which occur in wastewater pipework has led to various investigations and approaches to reduce microbial bioburden in hospital washbasin and sink drains (La Forgia *et al*., 2010). The replacement of fixtures and/or associated pipework is ineffective as new washbasins and pipework rapidly become recolonised with microorganisms (Vergara-López *et al*., 2013; Leitner *et al*., 2015; Wendel *et al*., 2015; Stjärne Aspelund *et al*., 2016; De Geyter *et al*., 2017). Disinfectants may fail to penetrate

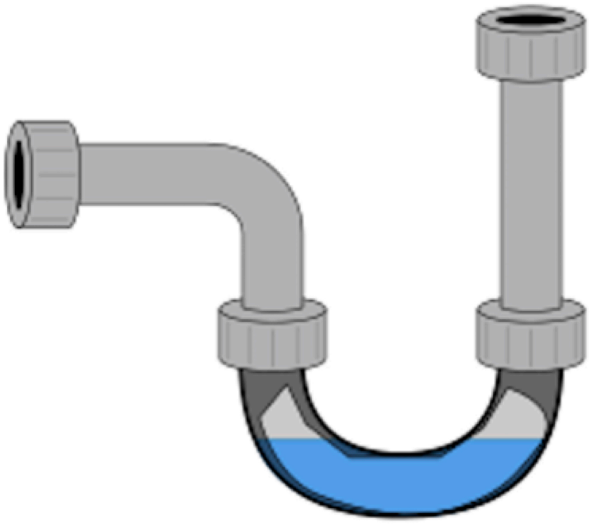

Figure 1.4 Schematic diagram of a washbasin U-bend. The vertical pipe in the upper right hand portion of the figure connects directly to the underside of the washbasin drain outlet. The horizontal pipe shown in mid-left hand portion of the figure connects to the wastewater pipe. A small volume of water is retained in the U-shaped portion of the U-bend (represented by blue shading), which acts as a seal preventing foul gases entering the facility housing the washbasin.

Figure 1.5 Photograph showing a washbasin drain outlet contaminated with visible biofilm. It can be clearly seen that the biofilm from the U-bend reaches the sink drain outlet.

dense biofilms and only temporarily damage their surface layers, necessitating regular and repeated use (Roux *et al*., 2013). Additionally, disinfectants poured down drains do not contact all areas of the pipework and may have short residency times in frequently used basins. A number of studies using bleach to control microbial contamination of washbasin drains and U-bends have been reported. La Forgia and colleagues (2010) developed a sink flushing protocol to control an *Acinetobacter baumannii* intensive care unit (ICU) outbreak involving flushing a gallon of diluted bleach through each sink's wastewater outlet and U-bend regularly. Although effective in controlling the outbreak, this approach was labour intensive, requiring healthcare workers to dilute and handle large volumes of bleach, which also had to be stored on site. Cholley *et al*. (2008) commented that although the daily use of bleach appeared effective it would be prudent to assess its efficacy over the long-term. Another approach involved integration of heating elements into U-bends, heating the wastewater to ≥ 85°C, followed by vibrational cleaning. This approach was reported as being effective over a 13-month study period (Fusch *et al*., 2015). However, U-bend water heating had to be activated when water temperature dropped to 75°C or when new water entered the U-bend, which incurred significant energy costs. It has also been suggested that the use of copper pipework in sink wastewater drains may exhibit higher antimicrobial activity than commonly used polyvinylchloride (Soothill, 2016). However, other research found that copper failed to sustain this effect in the long-term due to oxidation and mineral scale build up on pipe surfaces (Waines *et al*., 2011).

1.6 The root cause of the problem

Biofilm coats all of the moist internal surfaces of wastewater pipes but conventional disinfection involves pouring disinfectant down the washbasin drain, with no guarantee that the pipework above the U-bend has had adequate contact by the chemicals used. Secondly, the biofilms harboured in U-bends and associated pipework can be particularly dense and are not only comprised of bacteria but also consist of the slimy extracellular

polysaccharide matrix they produce as well as materials (hair, food, skin cells and other debris) washed down the drain. Moreover, washbasins should be used for washing hands only, but this is frequently not adhered to. Effective decontamination of any device or system first requires cleaning to reduce the amount of organic material present and then disinfection to reduce the amount of residual microorganisms (Dancer, 2014).

1.7 Electrochemically activated solutions

Over the last 10 years automated systems for minimising problematic microbial contamination of dental unit waterlines (DUWLs) and their associated water supply networks have been developed by the Dublin Dental University Hospital (DDUH), by treating supply water with pH-neutral anolyte solution (O'Donnell *et al*., 2009; Boyle *et al*., 2010; O'Donnell *et al*., 2011). Electrochemically activated (ECA) solution generators produce two solutions through activation of dilute brine, a metastable oxidant solution termed 'anolyte' (predominantly hypochlorous acid (HOCl)) and a second solution termed 'catholyte' with detergent properties (predominantly sodium hydroxide (NaOH)) (O'Donnell *et al*., 2009). The solutions are generated by passing a brine solution through a flow-through electrolytic cell (Figure 1.6). Anolyte is highly microbiocidal and capable of penetrating biofilms. The results of long-term studies (> 2 years) robustly demonstrated that the use of anolyte as a residual disinfectant consistently minimises microbial contamination of DUWLs and the output water (O'Donnell *et al*., 2009; Boyle *et al*., 2010; O'Donnell *et al*., 2011). More recently it was shown in another long-term (one year) study, that microbial contamination of clinical washbasin output water (both hot and cold) and associated taps can consistently be minimised by residual treatment of supply water with anolyte (Boyle *et al*., 2012).

Electrochemically activated solution (ECA) technology was originally developed in Russia in the 1970s (Bakhir, 1997; Bakhir *et al*., 2001; Bakhir, available at: http://www.bakhir.com/publications). Electrochemically activated solutions are produced

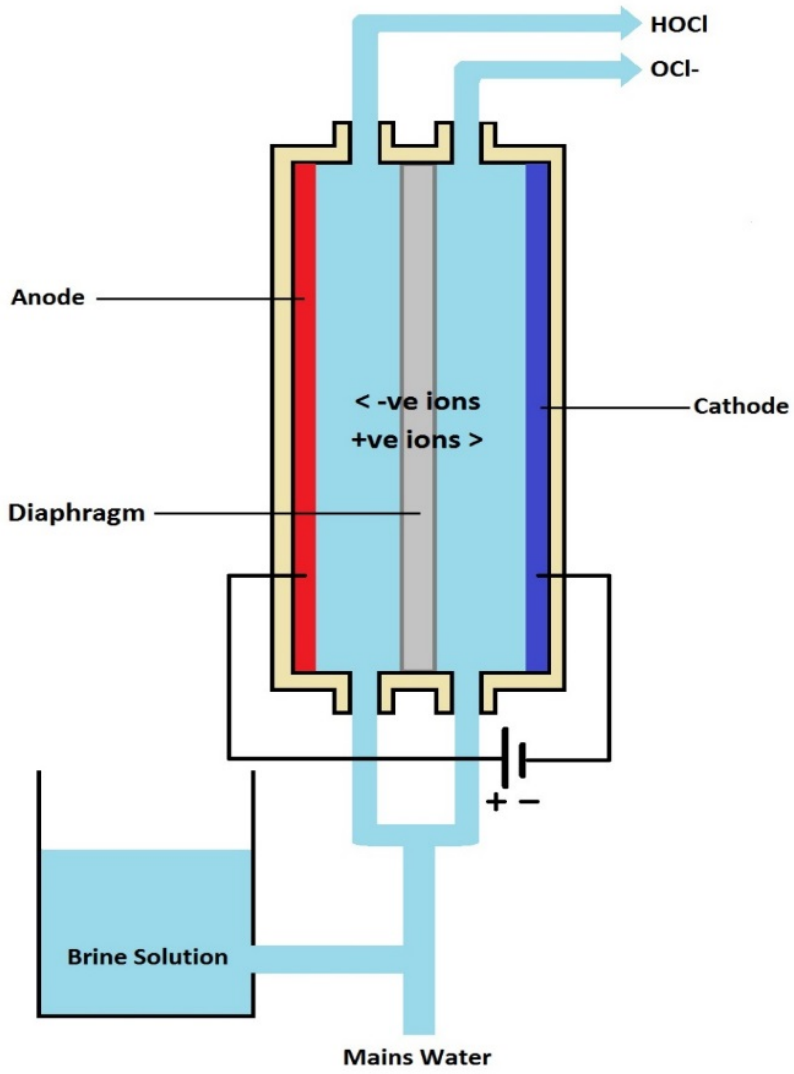

Figure 1.6. Schematic diagram outlining the generation of electrochemically activated solutions from a dilute salt solution.

by passing a dilute brine solution through an electric field in a Flow-through Electrolytic Module (FEM) cell and segregating the ions generated by the process, resulting in the production of two oppositely charged solutions with altered physical and chemical properties (O'Donnell *et al*., 2009). Electrochemical activation changes the state of the salt solution from a stable to a metastable state (Bakhir, 1997; Bakhir *et al*., 2001; Bakhir, available at: http://www.bakhir.com/publications). The positively charged oxidant solution (termed anolyte) typically has an oxidation-reduction reaction (redox) value of +600 mV, and consists of a mixture of unstable mixed oxidants (predominantly hypochlorous acid) in a physically excited state which is highly microbicidal and capable of penetrating microbial biofilms. The negatively charged antioxidant solution (termed catholyte) has detergent like properties, typically a pH of 11-13, a redox value of -600 mV and consists predominantly of sodium hydroxide. Electrically and chemically active microbubbles of electrolytic gas, 0.2 to 0.5 µm in diameter, are also generated during the activation process, which enhance the redox potential of the ECA solutions generated (Bakhir, 1997; Bakhir *et al*., 2001; Bakhir, available at: http://www.bakhir.com/publications; O'Donnell *et al*., 2009).

Electrochemically activated solutions can be produced by different types of ECA generators, each with particular properties and applications (Bakhir, 1997; Bakhir *et al*., 2001; Bakhir, available at: http://www.bakhir.com/publications). Several generations of FEM cells have been developed over the years, the FEM-3 being one of the more recent (Bakhir and Zadorozhny, 1997). ECA solution production with consistent properties was difficult to achieve prior to FEM-3 technology. FEM-3-based ECA generators can be configured to generate anolyte with a neutral pH, very much in contrast to anolyte produced by earlier ECA generators, which was often acidic (O'Donnell *et al*., 2009; O'Donnell *et al*., 2011).

1.7.1 The use of anolyte as a disinfectant

Electrochemically activated solutions have been used extensively in Russia for more than three decades, for drinking water disinfection, swimming pool disinfection, as a disinfectant in hospitals, for irrigating wounds, as inhaled sprays and many other infection control applications (Bakhir, available at: http://www.bakhir.com/publications). Early work on disinfection with anolyte solutions used ECA generators that produced inconsistent and acidic anolyte solutions that were corrosive and could have adverse effects on some materials (Coleman *et al*., 2007; Coleman *et al*., 2009; O'Donnell *et al*., 2011).

A variety of ECA oxidant solutions, also referred to as superoxidised water, oxidized water or anolyte, have been used as a residual disinfectant to control biofilm formation in DUWLs and such solutions were shown to be very effective for this purpose (Marais and Brozel, 1999; Kohno *et al*., 2004; Martin and Gallagher, 2005; Clark *et al*., 2006; O'Donnell *et al*., 2009; Boyle *et al*., 2010). However, not all ECA solutions are the same and some can have potential to cause adverse effects on DUWLs and on dental instruments connected to them following long-term use if the parameters for ECA solution production are sub-optimal or if the product used is too concentrated or too acidic (O'Donnell *et al*., 2009; Boyle *et al*., 2010; O'Donnell *et al*., 2011).

For most dental chair units, controlling biofilm in DUWLs by periodic or residual chemical agent treatment is usually performed separately for each unit. Ensuring consistent good quality DUWL output water from every dental unit is labour intensive and time consuming in dental clinics equipped with large numbers of units (Tuttlebee *et al*., 2002; Coleman *et al*., 2007; O'Donnell *et al*., 2007; Coleman *et al*., 2009; O'Donnell *et al*., 2011). This requires strict adherence to DUWL cleaning/disinfection protocols and the quality of supply water and the cleanliness of the water distribution network need to be monitored regularly. A study by O'Donnell *et al*. (2009) from this laboratory described the development of an automated, centralised water treatment system using residual anolyte at

DDUH to simultaneously control both dental unit supply water quality and DUWL output water quality for 103 dental units. The design of the centralised system consisted of two interlinked components, the first of which involved automatic sequential processing of chlorinated mains water by particle filtration, activated carbon filtration, kinetic degradation fluxion (KDF) filtration and water softening by ion exchange. The chemical quality of the processed water was shown to be consistently better than the limits proposed for water for human consumption (Anonymous, 1998). Processed water was stored in a storage tank providing water to the hospital's 103 dental units by a recirculating ring main. The second component of the system consisted of automated treatment of processed water with pH neutral anolyte at 2.5 parts per million (ppm)/ml. The level of anolyte in the water network was maintained by a series of in-line probes and free available chlorine monitoring equipment. The microbiological quality of processed and anolyte-treated dental unit supply water and DUWL output water from 10 dental units tested weekly for a 100-week period revealed aerobic heterotrophic bacterial counts averaged <1 and 18.1 colony forming units (CFU)/ml, respectively. These findings correlated with the absence of biofilm in DUWLs as determined by electron microscopy of the internal lumens of DUWL samples. No adverse effects due to anolyte treatment of supply water were observed for DUWLs or dental instruments supplied with anolyte-treated water during the study (O'Donnell *et al*., 2009). Similar results were achieved in a follow up study by Boyle *et al*. (2010) undertaken over a 60-week period with 10 dental units. The automated system required minimal human intervention, was environmentally friendly, did not yield harmful waste products, did not require the handling or storage of hazardous chemicals and yielded significant savings in operational costs, time and equipment down-time compared to individual disinfection of DUWLs in dental units.

Contaminated washbasin taps and output water are an important source of bacteria responsible for nosocomial infection. Another study from this laboratory by Boyle *et al*.

(2012) showed that residual treatment of hand washbasin tap supply water with residual pH 7.0 anolyte at 2.5 ppm can effectively minimise microbial contamination of both hot and cold output water. The study monitored the microbiological quality of output water from five sets of washbasin taps for a 54-weeks period. The mean counts of aerobic heterotrophic bacteria for washbasin hot and cold water and mains water during the study period were 1±4 CFU/ml, 2±4 CFU/ml and 205±160 CFU/ml, respectively. The majority of swab samples (33/40) from taps yielded no bacterial growth, while the remainder yielded less than present in mains water. No adverse effects due to anolyte were observed in the water network components, including taps (Boyle *et al*., 2012).

1.7.2 Safety of anolyte as a disinfectant

There are very few quantitative scientific studies outside of Russia that support or challenge the safety of anolyte for human exposure (Bakhir, available at: http://www.bakhir.com/publications). A variety of free radicals are generated during electrochemical activation of dilute salt solutions that may potentially have harmful effects following prolonged exposure or following exposure to very concentrated solutions (O'Donnell *et al*., 2009). It is important to emphasise that the limited exposure of dental patients and staff to the small quantities of free radicals in residual anolyte solutions in DUWL output water should not pose any health risk. Mammals have evolved complex anti-oxidant defence and repair systems to cope with natural exposure to hypochlorous acid (the principal ingredient of anolyte) and peroxides generated in inflammatory states by neutrophils and macrophages, whereas in contrast, microorganisms generally do not (McKenna and Davies, 1988; Wang *et al*., 2007).

A study by Boyle *et al*. (2010) investigated the cytoxicity of anolyte using cultured TR146 human keratinocyte monolayers and reconstituted human oral epithelial (RHE) tissue. Keratinocytes and RHE tissues were treated with anolyte (2.5-100 ppm) for 1 h periods after removal of growth medium and washing with phosphate buffered saline

(PBS). Similar experiments were undertaken using anolyte that had been exposed for 30 min to 1-2 μg/ml bovine serum albumin (BSA), equivalent to protein concentrations present in saliva. The Alamar Blue proliferation assay (which assesses cell viability) and the Trypan Blue exclusion assay (which assesses plasma membrane integrity) were used to quantitatively assess cytotoxic effects on monolayers following anolyte exposure. Cytotoxic effects on RHE tissues were assessed by the Alamar Blue assay and by histopathology. The study findings revealed that anolyte at >5.0 ppm resulted in significant ($P<0.001$) cytotoxicity to keratinocyte monolayers following a 1 h exposure. However, cytotoxicity was completely prevented by pretreatment of anolyte with BSA at concentrations equivalent to the protein levels usually found in human saliva. No cytotoxicity was observed in the more complex RHE tissue at anolyte concentrations up to 100 ppm (Boyle *et al*., 2010) These findings demonstrated that anolyte present as a residual disinfectant in DUWL output water is very unlikely to have adverse effects on human oral tissues at levels effective in minimising microbial contamination in output water.

ECA solutions have also shown potential to treat infected diabetic ulcers with significantly shorter healing times and no adverse skin reactions reported (Dalla Paola *et al*., 2006).

1.8 Purpose of this study

Microbial biofilms can harbour many microbial species, many of which have been identified as agents of infection in the hospital environment. In particular, washbasin and sink U-bends, which are frequently stagnant for significant periods, are of particular concern. While many efforts have been made to resolve this problem, none have been satisfactory in the long term. Our previous success using residual anolyte treatment to manage microbial contamination of water networks prompted us to investigate whether

ECA solution technology could be adapted and applied to minimise microbial contamination in washbasin U-bends and drains with regular automated treatment.

The purpose of this study was to develop an effective automated disinfection system for traditional U-bends used at washbasins using two solutions generated by electrochemical activation of brine including the disinfectant anolyte (predominantly hypochlorous acid) and catholyte (predominantly sodium hydroxide) with detergent properties. Pilot studies demonstrated that sequential manual treatment of U-bends with catholyte as a cleaning agent, followed by anolyte as a disinfectant could consistently minimise microbial contamination. These findings support the belief that an automated system, configured in this manner, should be capable of removing this major element of infection risk from the healthcare environment.

Chapter 2 Materials and Methods

2.1 Materials and Methods

2.1.1 Chemicals and reagents

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

2.2 Measurement of free available chlorine

Free available chlorine (FAC) levels in anolyte were measured using a Hach Pocket Colorimeter II (Hach Company, Iowa, USA) and a Hach CL 17 Free Chlorine Analyser (Hach Company, Iowa, USA) according to the manufacturer's instructions (Boyle *et al*., 2010; Boyle *et al*., 2012).

2.3 Anolyte and catholyte solutions

Electrochemically activated solution generators produce two solutions through activation of dilute brine, a metastable oxidant solution termed 'anolyte' (predominantly hypochlorous acid (HOCl)) and a second solution termed 'catholyte' with detergent properties (predominantly sodium hydroxide (NaOH)). Anolyte is highly microbiocidal and capable of penetrating biofilms. The electrochemically activated solutions used in this study were generated on-site using an Ultra-Lyte® UL-75a ECA generator (Clarentis Technologies, Florida, USA) supplied with Broxo® 6-15 high purity NaCl (Akzo Nobel Functional Chemicals BV, Arnhem, The Netherlands) and mains water. These reagents were automatically mixed at a ratio of approximately 0.1% (w/v) salt to 99.5% (v/v) water and automatically fed in to the ECA generator flow through membrane type electrolytic cell. The UL-75a generator is capable of generating anolyte solutions within a broad range of titers and pH. In this study the generator was configured by in-house engineering personnel to produce anolyte at 450 ppm free available chlorine (FAC) at pH 7.0 and catholyte with 400 ppm NaOH. The UL-75a is maintained, serviced and calibrated by

DDUH maintenance personnel and all associated consumable components (e.g. salt and water), are replenished as required.

2.4 Development of prototype system for washbasin U-bend cleaning and disinfection

Six identical ceramic washbasins (Armitage Shanks, Staffordshire, United Kingdom) located in adjacent staff rest rooms at DDUH were included in the study. In this washbasin model, the washbasin wastewater outlets (sink/plug hole) were located directly underneath the tap water flow. The rest rooms are located on three floors directly above and below each other, with two rest rooms per floor, male and female. All rest rooms are in frequent use Monday-Friday 9 am to 5 pm. The location of the rest rooms in DDUH was away from hospital clinical areas and was deliberately chosen so as not to disrupt day-to-day hospital activities and to facilitate regular U-bend cleaning, disinfection cycles, and U-bend sampling. The study washbasins were initially fitted with new 4 cm conventional polypropylene U-bend traps (Marley Plumbing and Drainage, Kent, United Kingdom) (***Figure 2.1***). These were subsequently replaced with Multikwik polypropylene U-bends (Marley Plumbing and Drainage) with an inspection/cleaning port to facilitate sampling of the U-bend interior (Figure 2.2.). A 1.25-inch (3.17 cm) manual ball valve (Philmac, Hailsham, East Sussex, United Kingdom) was fitted to the wastewater outflow pipework 50 cm downstream of each washbasin U-bend (Figure 2.3). The purpose of the manual valve was to seal the wastewater outlet from each washbasin so that the U-bend and pipework connected to it could be completely filled with cleaning/disinfectant solutions to a level above the washbasin drain outlet.

The materials used in the U-bend and wastewater pipework were polypropylene and unplasticised polyvinyl chloride (uPVC), respectively. Unplasticised PVC is totally compatible with anolyte (1000 ppm at pH 7 to 8) and catholyte (at pH 11-13.5) (see

Appendix 1). Polypropylene is compatible with anolyte (1000 ppm between pH 7 to pH 8) but catholyte can cause minor damage at pH 11-13.5 (see Appendix 1). No evidence of any damage to the pipework was observed during the course of the present study. Three washbasins, one on each floor, were selected for ECA cleaning and disinfection of U-bends (i.e. test U-bends), while the other three adjacent washbasin U-bends were used as controls. Test U-bends were treated with freshly generated ECA solutions, whereas control U-bends were treated with mains water.

2.5 Pilot study of ECA treatment of U-bends

Preliminary cleaning and disinfection of test U-bends was undertaken manually. Initial experiments were performed with conventional U-bends (Figure 2.1). Preliminary experiments were undertaken with standard polypropylene U-bends that had to be detached from the washbasins by unscrewing the coupling ring that attached the U-bends to the underside of each washbasin wastewater outlet. Following swab sampling of the interior lumen of each U-bend, the U-bends were reattached to the washbasin wastewater outlets by re-screwing the coupling ring. This process was found to be unsatisfactory as on many occasions post-sampling the U-bends leaked at the point of attachment to the washbasin drain outlets. This was due to the repeated screwing and unscrewing of the U-bend from its housing. This process was also time consuming and awkward. Therefore, the U-bends on all six washbasins were replaced with polypropylene U-bends with an integral inspection port that could be used to facilitate swab sampling of the U-bend interior (Figure 2.2).

Manual ECA U-bend treatment cycles were initiated by turning the handle on the manual ball valve through 90°, thus sealing the wastewater outlet pipe downstream of the U-bend. Then freshly prepared catholyte solution (1:10 dilution with mains water) was manually poured down the washbasin drain outlet until the solution filled the washbasin to a level several centimetres above the drain outlet. The manual valve was opened partially during filling to reduce the occurrence of air being trapped in the U-bend and the

Figure 2.1 Photograph showing a conventional U-bend connected to the underside of a washbasin drain outlet. This washbasin and U-bend was one of three used for manual ECA U-bend decontamination in the initial phase of the study.

Figure 2.2 A photograph showing an example of a Multikwik polypropylene U-bend. This is the U-bend used with the automated ECA U-bend decontamination system developed in the present study. The U-bend is connected directly below the washbasin drain outlet. The sampling port visible in the upper right portion of the photograph can be unscrewed easily for taking swab samples.

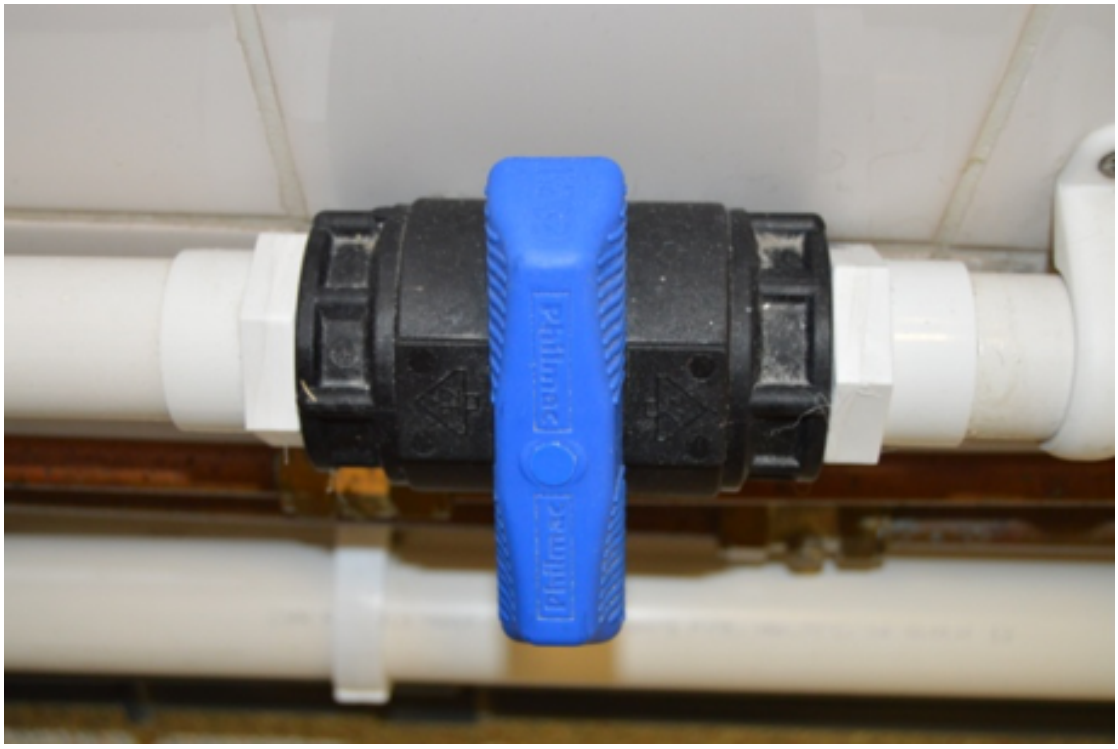

Figure 2.3 Photograph showing an example of one of the manual valves (shown in the closed position) fitted to the wastewater pipework downstream of a washbasin U-bend used in the manual disinfection part of the present study.

wastewater pipework connected directly to it and to ensure the U-bends were completely filled with catholyte solution. The manual valve was then closed leaving catholyte solution covering the washbasin drain outlet to a level of approximately 50 mm. The catholyte solution was left *in situ* for 5 min and then voided to waste by opening the manual valve. The manual valve was then closed again and the process repeated with undiluted anolyte solution. Anolyte solution was left *in situ* for 5 min and then voided to waste by opening the ball valve and purging the U-bends with cold tap water. Control U-bends were treated in the same way as the test U-bends using mains water instead of ECA solutions. Immediately following U-bend treatment, swab samples of the internal surfaces of each U-bend were taken for microbiological culture analysis. Preliminary experiments were undertaken with conventional U-bends without a sampling port (Figure 2.1). Sampling required unscrewing the securing rings at the top (connected to washbasin drain outlet) and bottom (connected to the wastewater pipe) of the U-bend. Swab samples were taken down the top throat of the U-bend. After sampling, the U-bend was reassembled and put back in service.

2.6 Automated ECA treatment system for U-bends

2.6.1 Initial design concepts

Following successful decontamination of U-bends by manually dosing the sinks with ECA solutions, efforts were focused on the possibility of automating the dosing cycle. One of the first floor rest rooms used in the pilot manual decontamination study was selected as the site for an automated prototype system with the adjacent bathroom to be the control. The initial concept for automated ECA treatment of U-bends was to replace the manual valve used on the 40 mm wastewater drain pipe in the pilot study with an electronic motorised actuator and interlinked ball valve. The purpose of the actuator is to provide the rotary motion to turn the ball valve permitting automated sealing of the wastewater pipe outlet during the process and allow the liquids remain in the U-bend and associated pipe

work upstream of the valve. This actuator would have to be sized to fit into the corresponding 40 mm diameter wastewater drainpipe.

As outlined above, the conventional U-bends leaked due to seal failure and were replaced with Multiwik U-bends (Figure 2.2) with an integrated cleaning port that was used to facilitate sampling of the U-bend interior.

Two reservoir containers to store anolyte and catholyte solutions for U-bend ECA treatment were required and would be filled with freshly generated catholyte and anolyte as required. Initially it was decided to mount the reservoirs on the wall above the test washbasin to allow the liquids to be gravity fed into the U-bend and adjacent pipework. The containers would each be separately connected to the wastewater pipework close to the U-bend using a flexible chemical resistant pipe. At the base of each of the reservoirs, a solenoid valve would be fitted to the outlet that would open and close when provided with an electronic signal. Opening the solenoid valve on the catholyte or anolyte reservoirs would permit the solution to be released under its own weight into the washbasin and pipework.

A Programmable Logic Controller (PLC) would be required to provide the logic and sequence the electronic signals between solenoid valves on the base of the catholyte and anolyte reservoirs and the actuator on the drain outlet. It was proposed that the system would be programmed to allow for automatic ECA treatment of the test washbasin U-bend at predetermined times through the PLC programme.

2.6.2 Initial design concept equipment

Two 10 L polypropylene containers (EDA Plastiques. Oyonnax, France) were used to each hold the anolyte and catholyte for dispensing the liquids into the washbasin U-bend and associated pipework. Each of the polypropylene containers was mounted inside a fabricated steel container mounted on the wall 150 cm above the test washbasin selected

for automated ECA treatment. Two Hydralectric solenoid valves (Figure 2.4) (Hydralectric Group, Surrey, England) were fitted on the outlet of each of the containers. These valves are used in low-pressure water dosing systems, have a polyamide body and are approved for potable water use under the Water Regulation Advisory Scheme (WRAS UK). They are normally closed solenoid valves used for controlling water at a minimum pressure of 0.2 bar.

The inlet port of the reservoirs were connected directly to one end of the 1.27 cm (½" British Standard Pipe (BSP)) screw thread outlet port on the solenoid valve. The outlet of the solenoid valve was connected to a 6 mm diameter PVDF (polyvinylidene fluoride) tube for each of the two ECA solution reservoirs. Two 6 mm tubes, one from the catholyte container and the other from the anolyte container, were funnelled through a 19 mm diameter braided hose for protection and to keep the pipes tidy in the rest room area. The braided hose was attached to the wall and fed down the side of the washbasin for dosing into the U-bend.

The 40 mm wastewater pipe work from under the washbasin drain outlet up to the inlet of the motorised valve was replaced with uPVC to provide support and strength for the motorised actuator and to provide a location for the dosing points. Two small holes were drilled in the pipework downstream of the U-bend and two nipples, for connection to the 6 mm PVDF tubing, were fitted into these holes. These nipple points were fitted at the lowest point on the system up stream of the actuator. This would allow any trapped air escape up through the U-bend and sink hole during filling with ECA solutions. As the liquid fills the system from below, air is displaced up through the pipe work and U-bend. The nipples were glued in place using silicon and allowed to dry. Then the two 6 mm diameter tubes from the ECA reservoirs were each attached to one of the nipples providing the pathway for delivery of ECA solutions to fill the pipework and U-bends.

The manual valve on the wastewater outlet pipe downstream was replaced with a Vexve Termomix D32 ball valve (Sastamala, Finland) fitted with an electronic Joventa 16Nm standard actuator (Figure 2.5) (Bratislava, Slovakia). This type of actuator has pre-set limit switches that restrict the actuator from over rotating and can only be maintained in the open or closed position. This actuator provided the rotary power to turn the ball valve through a 90° angle only, open and closed.

An Open System Solutions (Hampshire, United Kingdom) model NeOSS-V3-16A-MP open protocol programmable logic controller (PLC) was used to provide the logic behind the sequence of operations for automated U-bend treatment with ECA solutions.

2.6.3 Initial design concept operation

The respective ECA reservoirs were filled with anolyte and catholyte. The catholyte container was filled with one litre of catholyte solution at 400 ppm and nine litres of mains potable water. The anolyte solution was filled directly from the ECA generator at 450 ppm. The screw caps on the reservoirs were tightened and the air vent left slightly open. The reservoirs were then placed into the steel wall containers erected on the wall and then connected to the inlet side of the solenoid valve attached to the reservoir outlets.

The sequence of operation was similar to the manual disinfection system (Figure 2.6). On commencement of the cycle, the actuator rotated and closed the ball valve. The solenoid at the outlet of the catholyte container was opened and the catholyte solution was released into the pipe upstream of the U-bend. The liquid flowed under gravity into the pipe and gradually filled the pipe, U-bend and washbasin. When the catholyte rose to 50 mm above the washbasin drain outlet, the solenoid valve at the base of the catholyte reservoir was closed. This time was recorded and later programmed into the PLC for future cycles. The catholyte was allowed to remain in the U-bend and associated pipework for 5 min. After this time, the actuator was activated to open the ball valve discharging the

Figure 2.4 A photographic showing a Hydralectric Solenoid Valve 72003 used in the first pilot study. This valve is Water Regulations Advisory Scheme (WRAS) approved (https://www.wras.co.uk/). The two electrical connections at the front are operated via a programmable logic controllor (PLC), which opens and closes the valve. This valve operated inconsistently due to pressure reductions as the volume of solutions in the ECA reservoirs decreased following initial rounds of ECA U-bend decontamination.

Figure 2.5. **Photograph showing the electronic Joventa actuator and Vexve ball valve fitted to the wastewater pipe downstream of the U-bend of the test washbasin used for automated ECA U-bend decontamination.** This opens and closes the ball valve in the automated U-bend cleaning and disinfection system developed in the present study.

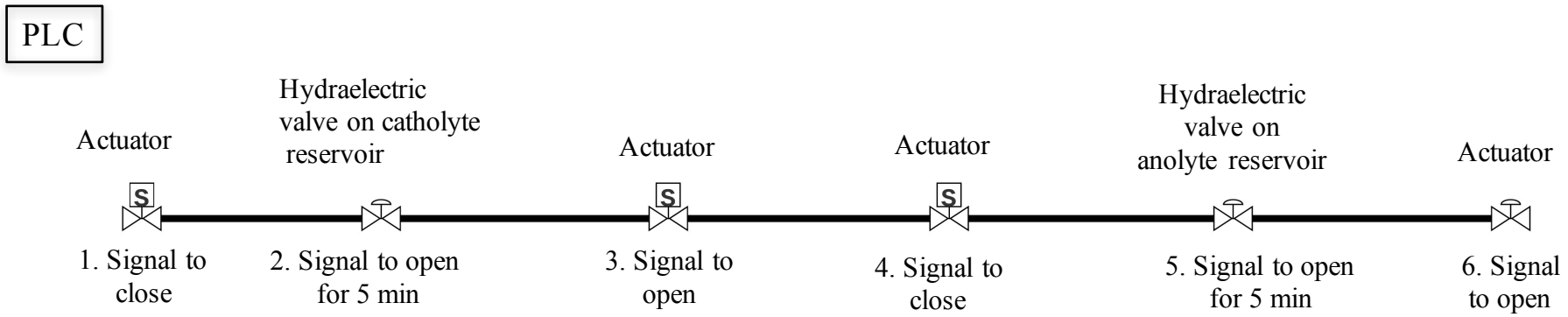

Figure 2.6 Process control diagram for the programmable logic controller (PLC) used with the initial prototype automated ECA treatment system for U-bend decontamination. The PLC operates control relays with a 24 V direct current output. The process starts with the PLC sending the first signal (1) to the actuator to close the ball valve on the washbasin wastewater outlet. Following a 20 s delay the PLC sends a signal (2) to the hydraelectric valve on the outflow from the catholyte reservoir releasing catholyte into the washbasin U-bend and associated pipework. Catholyte is left *in situ* for 5 min after which another signal (3) from the PLC to the actuator opens the ball valve on the washbasin wastewater outlet voiding spent catholyte to waste. Then another signal from the PLC (4) closes the actuator. Following a 20 s delay the PLC sends another signal (5) to the hydraelectric valve on the outflow from the anolyte reservoir releasing anolyte into the washbasin U-bend and associated pipework. Anolyte is left *in situ* for 5 min after which another signal (6) from the PLC to the actuator opens the ball valve on the washbasin wastewater outlet voiding spent anolyte to waste.

catholyte into the wastewater stream. The actuator was allowed to rest for 20 s after which the actuator was rotated again, closing the ball valve on the wastewater outlet pipe. The solenoid valve at the base of the anolyte reservoir was then opened, releasing anolyte into the pipework, U-bend and washbasin a level approximately 50 mm above the washbasin drain outlet. This time was also recorded and programmed into the PLC. Anolyte was left *in situ* for 5 min and then voided to the wastewater stream by opening the ball valve, completing the cycle.

2.6.4 Problems encountered with initial design concept operation

The first automated ECA U-bend treatment cycle with full ECA reservoirs was successful. However, problems were encountered with subsequent cycles as the ECA reservoirs began to empty. Fill times were erratic and in some cases, insufficient liquids were dispensed into the U-bends and pipework. This happened on both the catholyte and the anolyte dispensing cycles. After repeating the cycles with full and partially full ECA reservoirs, it became evident that the flow varied due to the head of pressure in the reservoir. As the pressure decreased in the reservoirs as the reservoirs emptied, the ECA solution was not able to overcome the minimum pressure needed to open the hydrostatic pressure of the solenoid valve. The cycle programmed in the PLC to fill the U-bend was based on time and as the flow rate was linked to pressure, the quantity of liquid being dispensed into the U-bend and pipework was inconsistent. An alternative means of delivering ECA solutions was necessary.

2.6.5 Secondary design concept process

It was decided that the problems encountered in the initial design process with flow and pressure could be overcome by using a variable flow pump system for delivering ECA solutions to the washbasin U-bend and pipework with minimum pressure on the outlets of the catholyte and anolyte reservoirs. This pump system would interface between the outlet of the reservoirs and the inlets to the pipe system. Two pumps were required, one for each

of the ECA solutions. Chemical resistant TEKNA EVO TCK 603 (Figure 2.7) (Rieti, Italy) pumps were selected as they contain a fluoro-rubber (FPM) seal, a polyvinylidene fluoride (PVDF) pump head and a polytetrafluoroethylene (PTFE) diaphragm making them ideal for chemical dosing. Anolyte at 1000 ppm and catholyte at pH 13 have no adverse effects on FPM, PVDF and PTFE materials (Appendix 1). This pump also contains a digital timed solenoid permitting relatively simple integration into the PLC programme. A 6 mm diameter polyvinyl chloride (PVC) tube from the inlet side of each pump was placed into the top of the respective container and the polyethylene (PE) delivery tube was connected from the outlet side of the pump to the existing nipples, used on the initial design process (see section 2.6.2), at the dosing points on the wastewater pipe below the washbasin and upstream of the U-bend.

The pumps were initially mounted in the same cabinets as the catholyte and anolyte reservoirs described above (see section 2.6.2). However, as the concept of gravity feed of ECA solutions envisaged in the initial design concept was now redundant, it was decided to relocate the ECA reservoirs and pumps to a more convenient location in a lockable cabinet at ground level adjacent to the test washbasin (Figure 2.8). This facilitated access to the pumps and ECA reservoirs and avoided potential safety hazards associated with filling ECA reservoirs located 1.5 m above the washbasin.

The time to fill the pipe work, U-bends and washbasin to a level 50 mm above the washbasin drain outlet was recalculated using the dosing pumps. The volume of liquid required to fill these areas was determined empirically (approximately 700 ml). The optimum time using the dosing pumps to fill the pipework, U-bend and washbasin with ECA solution was determined as 5 min. This provided enough time for the ball valve to open, close and rest and for the dosing pump to fill the required areas. Having determined these parameters, the PLC programme could be finalised and the set points were inputted into the PLC controller (Figure 2.9). This was achieved using a Lenovo Think Pad Type

Figure 2.7 Photograph showing an example of a chemical resistant TEKNA EVO TCK 603 chemical resistant pump used in the second prototype automated ECA decontamination system. Two dosing pumps of this type were used with the system to pump ECA solutions into the test U-bend and associated wastewater pipework. A separate pump was used for each of the cathoylte and anolyte solutions used. Chemical resistant TEKNA EVO TCK 603 (Rieti, Italy) pumps were selected as they contain a fluoro-rubber (FPM) seal, a polyvinylidene fluoride (PVDF) pump head and a polytetrafluoroethylene (PTFE) diaphragm making them ideal for chemical dosing. Anolyte at 1000 ppm and catholyte at pH 13 have no adverse effects on FPM, PVDF and PTFE materials (Appendix 1). This pump also contains a digital timed solenoid permitting relatively simple integration into the programmable logic controller (PLC) programme.

Figure 2.8 Photograph showing the two Tekna dosing pumps and catholyte and anolyte reservoirs located in a locakable cabinet at floor level adjacent to the test washbasin used for automated ECA U-bend decontamination in the present study.

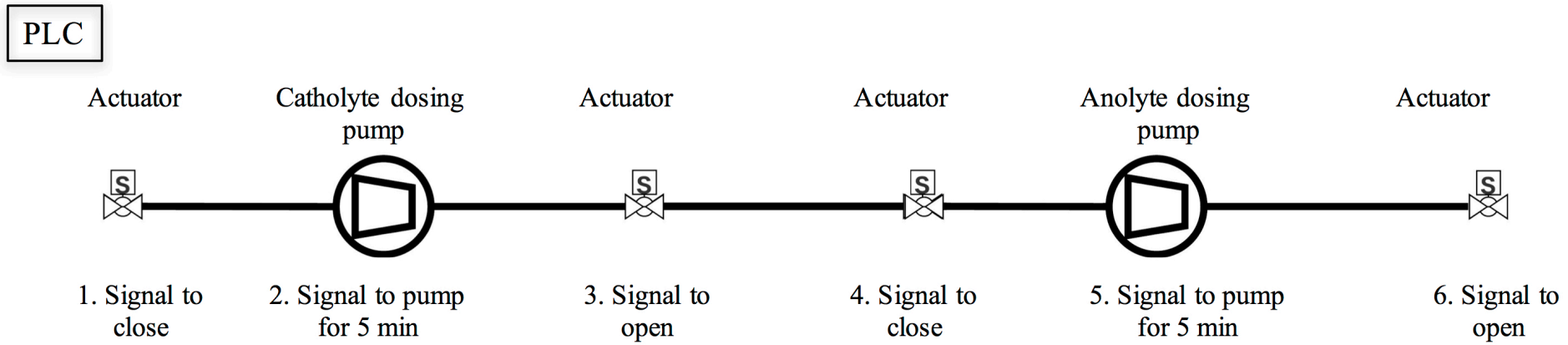

Figure 2.9 Process control diagram for the programmable logic controller (PLC) used with the second prototype automated ECA treatment system for U-bend decontamination. The PLC operates control relays with a 24 V direct current output. The process starts with the PLC sending the first signal (1) to the actuator to close the ball valve on the washbasin wastewater outlet. Following a 20 s delay the PLC sends a signal (2) to the catholyte pump which pumps catholyte from the catholyte reservoir into the washbasin U-bend and associated pipework. Catholyte is left *in situ* for 5 min after which another signal (3) from the PLC to the actuator opens the ball valve on the washbasin wastewater outlet voiding spent catholyte to waste. Then another signal from the PLC (4) closes the actuator. Following a 20 s delay the PLC sends another signal (5) to the anolyte pump which pumps anolyte from the anolyte reservoir into the washbasin U-bend and associated pipework. Anolyte is left *in situ* for 5 min after which another signal (6) from the PLC to the actuator opens the ball valve on the washbasin wastewater outlet voiding spent anolyte to waste.

4291-M52 (version 2.6.4) (Singapore) and connecting directly to the PLC using a CAT 5 serial cable.

2.6.6 Secondary design concept operation

The prototype automated ECA treatment system for washbasin U-bends was tested extensively for operation and malfunctions over several weeks. Having optimised the system parameters, automated U-bend ECA treatment cycles were commenced. 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.

2.7 Sampling of U-bends

Immediately following each ECA treatment cycle, the interior surface of the U-bends from the test and control washbasins were sampled using sterile cotton wool swabs (Venturi, Transystem, Copan, Italy). Immediately prior to sampling, swabs were dipped in sodium thiosulphate (0.5% w/v) solution before use to neutralise residual FAC as described previously (Boyle *et al*., 2010; Boyle *et al*., 2012) and were processed immediately.

2.8 Microbiological culture of U-bend samples

The tip of each swab 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) (Swan *et al*., 2016). 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 (Reasoner and Geldreich, 1985; Boyle *et al*., 2010; Boyle *et al*., 2012). The inclusion of sodium pyruvate in R2A medium also leads to enhanced recovery of chlorine stressed bacteria (Reasoner and Geldreich, 1985; Calabrese and Bissonnette, 1990; Boyle *et al*., 2010; Boyle *et al*., 2012).

Bacterial 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.

2.9 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.

2.9.1 Bacterial culture and storage

Isolates were selected from CBA plates and single colonies were inoculated onto Trypticase Soy Agar (TSA) and incubated overnight (18 h) in a static incubator (Gallenkamp, Leicester, UK) at 37^{o}C. This was repeated until the culture was pure. For

long term storage, isolates were stored at -70^{o}C in individual preserver vials (Protect Bacterial Preservation System, Technical Services Ltd., UK).

2.9.2 Whole-genomic DNA extraction

Whole genomic DNA was extracted from each isolate using the DNeasy Blood and Tissue Kit (Qiagen, Crawley, UK) as described previously (O'Donnell *et al*., 2006). Each isolate was cultured by removing a single bead from the individual preserver vial using a sterile inoculating wire loop and inoculating a TSA plate. Plates were incubated in a static incubator at 37^{o}C overnight. A single isolated colony was selected and lawned onto a fresh TSA plate using a sterile wire loop and incubated for 18 h in a static incubator at 37^{o}C. The cells were then lysed by adding a 2.5 cm^{2} area of culture growth from a lawned TSA plate using sterile bacteriological loops (Greiner Bio-One GmbH, Germany) into 200 µl of lysis buffer (5 ml of TE buffer and 0.02 mg lysozyme). This was incubated for 2-3 h at 37^{o}C with shaking (250 rpm). Following lysis, the DNeasy Blood and Tissue kit was used according to the manufacturer's instructions. The addition of 25 µl of proteinase K and 200 µl of buffer AL (both supplied with kit) and incubation at 70^{o}C for 30 min removed proteins and nucleases, respectively. Released genomic DNA was then bound to a silica gel membrane inside a mini-column (supplied with kit). The membrane was washed twice with buffers that were supplied with the kit. These buffers contained ethanol and salt and were used to wash cellular debris from the column but leaving the DNA bound to the membrane. The DNA was finally eluted into 200 µl of elution buffer with centrifugation at 15, 970 x g for 1 min using an Eppendorf model 5417C centrifuge fitted with an F-45-30-11 rotor (Eppendorf, Hamburg, Germany). The DNA was stored at 4^{o}C (for storage of four to six weeks) or at -20^{o}C (for long-term storage). The concentration of genomic DNA (ng/µl) was measured using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc, Massachusetts, USA). The quality of DNA was assessed by conventional agarose gel electrophoresis in 0.8% (w/v) agarose gels.

2.9.3 Species identification of isolates

Species identification of all isolates was performed by PCR amplification and sequencing of the small ribosomal subunit rRNA gene sequences using previously published primers 533F (5'-AGAGTTTGATYMTGGCTCAG-3') and 142-R (5'-CGGYTACCTTGTTACGAC-3') (Singh *et al*., 2003). The following thermocycling conditions were used: 94^{o}C for 2 min, followed by 35 cycles of 94^{o}C for 30s, 50^{o}C for 30s and 72^{o}C for 10s, and a final elongation step of 72^{o}C for 10 min. The amplimers were sequenced commercially by Source Biosociences (Tramore, Waterford, Ireland) using an ABI 3730x Sanger sequencing platform. DNA sequences were analysed using BioNumerics software package (version 7.6) (Applied Maths, Belgium) and BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al*., 1990).

2.10 Electron microscopy of U-bend samples

Following the completion of automated treatment cycles, the test and control U-bends were removed and cut longitudinally through the lumen and the internal walls examined for the presence of biofilm, without prior fixation, by electron microscopy using a Zeiss Supra 35 variable pressure field emission scanning electron microscope as described previously (Tuttlebee *et al*., 2002; O'Donnell *et al*., 2006; O'Donnell *et al*., 2007). All electron microscopy was undertaken commercially by the Centre for Microscopy and Analysis (CMA), University of Dublin, Trinity College, Dublin, Ireland.

2.11 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.).

Chapter 3 Results

3.1 Results

3.2 Manual U-bend treatment with ECA solutions

The concept of U-bend decontamination with ECA solution was investigated initially using a manually operated prototype system with three test washbasins to establish proof of concept. Three identical washbasins were used as controls. The prototype system is described in detail in the Materials and Methods, Section 2.4. In brief, the manual system consisted of installing a manual ball valve on the wastewater outflow pipe of each test washbasin (Figure 3.1). Turning the valve in a clockwise direction sealed the outflow pipe.

Manual U-bend treatment with ECA solutions was initiated by closing the ball valve (Figure 3.1) and pouring freshly generated catholyte solution down the washbasin drain until the liquid covered the drain outlet. The ball valve was then opened partially to allow catholyte solution to fill the U-bend and pipework as far as the ball valve with catholyte. The volumes of catholyte required for this purpose were determined empirically. Then the valve was closed and additional catholyte poured into the drain to a level several centimetres above the washbasin drain outlet. Catholyte was left *in situ* for five min and then voided to waste by opening the ball valve. The same procedure was repeated with freshly generated anolyte solution. Following anolyte treatment, the washbasin was flushed with tap water and the U-bend unscrewed from below the washbasin (Figure 2.1) and swab samples were taken from the interior with swabs dipped in neutralisation solution. These swabs were then transported immediately to the Microbiology Laboratory and processed.

Microbiological sampling of the three control washbasin U-bends tested once weekly for five consecutive weeks showed all were heavily contaminated with bacteria. The mean average bacterial density on CBA and R2A agars was 2.41×10^5 ($\pm 2.5 \times 10^5$) and 1×10^6 ($\pm 9.9 \times 10^5$) CFU/swab, respectively, (CBA range 4.8×10^3 - 7.6×10^5 CFU/swab; R2A range 9.2×10^3 - 3.8×10^6 CFU/swab). Figure 3.2 shows a cross-section

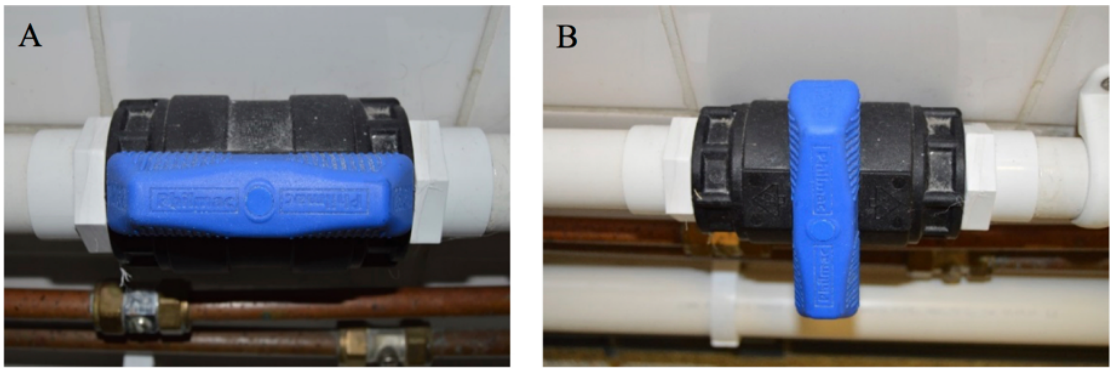

Figure 3.1 Photograph showing a manual valve fitted to a test washbasin wastewater outflow pipe downstream of the U-bend. In position (A) the valve is open and allows liquid to flow. In position (B) the valve is in the closed position and prevents liquid flowing.

Figure 3.2 Photograph showing a cross-section of a U-bend from one of the control washbasins after the five-week initial study period. Extensive heavily pigmented microbial biofilm is evident throughout the U-bend, including areas above and below where water is retained.

of a U-bend from a control washbasin after the initial five-week study period. Figure 3.3 shows a CBA agar plate following inoculation with a swab from a control U-bend following 24 h incubation.

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.

3.3 Development of prototype automated ECA U-bend treatment system

3.3.1 Initial automated system design

For automated U-bend disinfection, one washbasin was used as the test washbasin and one washbasin used as the control unit. The existing U-bends were replaced with U-bends with an integrated inspection/cleaning port to facilitate sampling of the U-bend interiors without having to unscrew the U-bends from their housings below each washbasin (see Figure 2.2). For the test washbasin, the manual ball valve on the wastewater outflow pipe was replaced with an electrically operated ball valve known as a solenoid actuator and ball valve (see Figure 2.5). Automated dosing of ECA solutions into the test washbasin U-bend was initially developed using a gravity feed system to dispense ECA solutions into the test U-bend and associated pipework. For this purpose, two 10-L polyethylene containers were mounted on the wall above the test washbasin to supply catholyte and anolyte solutions. A hydralectric solenoid valve was fitted at the base of each reservoir, which provided the mechanism to open and close the outlet of each container. The reservoirs and hydraletric valve were housed inside pressed steel lockable panels mounted on the wall above the test

washbasin. The outlets of the solenoid valves were fitted with polyvinylidene fluoride flexible tubing that directed the ECA solutions from the reservoirs into the washbasin pipework and U-bends. The flexible tubing was connected at separate points to a 20 mm acrylonitrile-butadiene-styrene pipe connected below the washbasin U-bend upstream of the actuator. The connection points were positioned at the lowest point on the pipework of the area being filled to avoid any air being trapped within the system while filling. A programmable logic controller (PLC) sequenced the operation of the hydraletric valve and actuator. The PLC allowed the timing, duration and sequence of activation of the actuator and pumps to be programmed. The system is outlined schematically in Figure 2.6.

3.3.2 Testing of initial automated system design

The ECA treatment cycle was initiated within the electrical panel by overriding the PLC input. This was initially done to test the cycle operation of the system and would be replaced later by timed controls with the PLC. When the cycle was initiated, the actuator rotated in turn closing the ball valve on the wastewater outflow pipe. Following a 20 s delay, the hydraletric valve opened at the base of the full catholyte reservoir and began to fill the pipework and U-bend. During this process, catholyte slowly filled the U-bend from below and caused trapped air and water from the U-bend to rise into the washbasin through the washbasin drain outlet. The hydralectric valve closed when the catholyte reaches 50 mm above the washbasin drain, this was initially achieved by recording the time and programming it into the PLC. The catholyte was left *in situ* for five minutes as dictated through the PLC and then the actuator rotated back to its original position in turn opening the ball valve, which voided the spent catholyte to waste. After a further 20 s delay, the actuator then closed the same ball valve again on the wastewater outlet pipe. Following another 20 s delay, the hydroelectric valve at the base of the full anolyte reservoir opened and dosed anolyte into the system and the cycle proceeded as per catholyte dosing. Anolyte

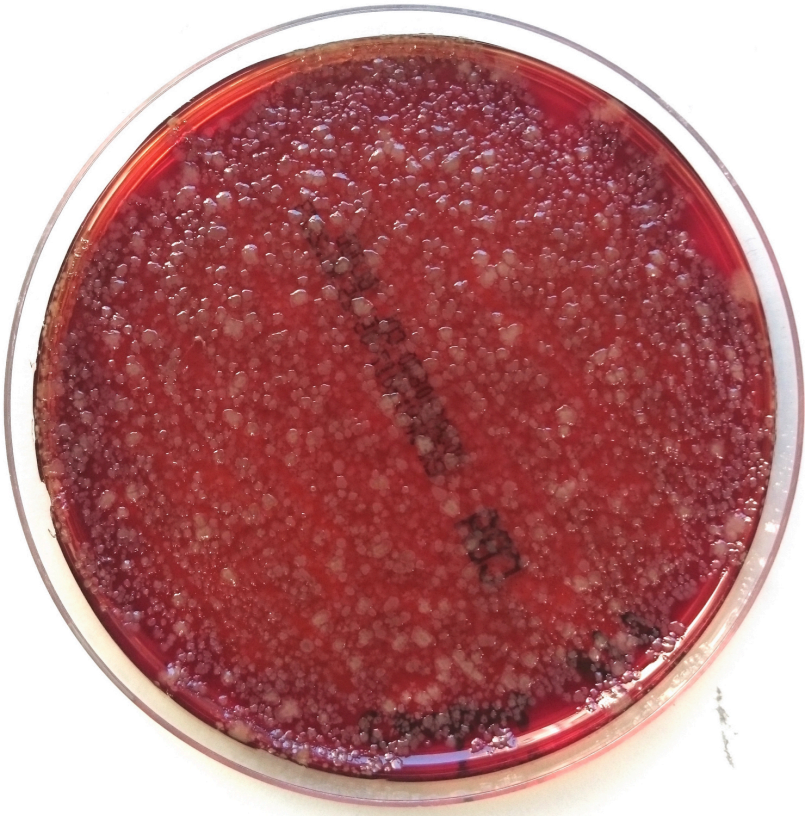

Figure 3.3 Photograph showing a CBA plate inoculated with a swab from a control washbasin U-bend showing extensive bacterial contamination following 24 h incubation at 37°C.

was left *in situ* for 5 min where the ball valve on the wastewater outlet would open and the spent anolyte voided to waste, completing the cycle.

The time taken to fill the washbasin to 50 mm above the drain outlet was recorded and programmed into the PLC. However, subsequent U-bend fill times were irregular and in some cases, no liquids were dispensed from the ECA reservoirs into the U-bend. The consistency of flow from the reservoirs into the pipework and washbasin U-bend following numerous tests was found to be erratic following repeated cycles of ECA treatment. The hydralectric solenoid valves at the reservoir outlets were rated 0.2 bar – 10 bar, however, it became evident that as the reservoirs emptied the pressure of liquid required to open the valves reduced. The gravity valves required to open on pressure modulated as the pressure reduced in the reservoirs causing an inconsistent flow and is some cases as the liquid levels dropped in the reservoirs the valves closed. A constant pressure in the reservoirs was therefore required to guarantee the flow output. It was evident that the use of gravity feed to dispense ECA solutions was unreliable given the fluctuations in pressure and subsequently flow. However, for the purposes of our prototype an alternative method of delivering ECA solutions from the reservoirs to the washbasin U-bend was employed.

3.4 Development of second prototype automated ECA U-bend treatment system

The same test washbasin was used for the second prototype system. The hydraletric solenoid coils on the ECA reservoirs were replaced with digital timed solenoid dosing pumps, one for the anolyte and one for catholyte. These dosing pumps ideally suited for swimming pool applications were selected as they contain a FPM fluoropolymer seal, a PVDF pump head and PTFE diaphragm making it ideal for chemical dosing. Anolyte at 1000 ppm and catholyte at pH13 cause no damage to FPM, PVDF and PTFE materials. To facilitate ease of filling with ECA solutions, the ECA reservoirs were repositioned in a

lockable cabinet adjacent to the test washbasin to house dosing pumps and the two 10-L reservoirs. Each reservoir supplied separate dosing pumps connected by 6 mm diameter polyvinylidene fluoride flexible tubing into the same connections below the washbasin U-bend. The dosing pumps replaced the hydraletric solenoid valve used in the initial automated prototype in the PLC programme. The system is outlined in Figures 2.9 and 2.11 (Chapter 2) and Figure 3.4. The mechanics and electrics in the final assembly were stored unobtrusively in the locked cabinet along with the ECA reservoirs (Figure 3.5). The catholyte and anolyte reservoirs were readily accessible for filling.

3.5 Automated ECA U-bend treatment using the second prototype system

Automated disinfection cycles were timed for 07.00 h and began with the actuator closing the valve on the wastewater outflow pipe. Following a 20 second 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.

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 disinfection cycle and the quantitative density of bacteria recovered determined on a variety of culture media. An identical 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 1×10^5 CFU/swab in each case (Table

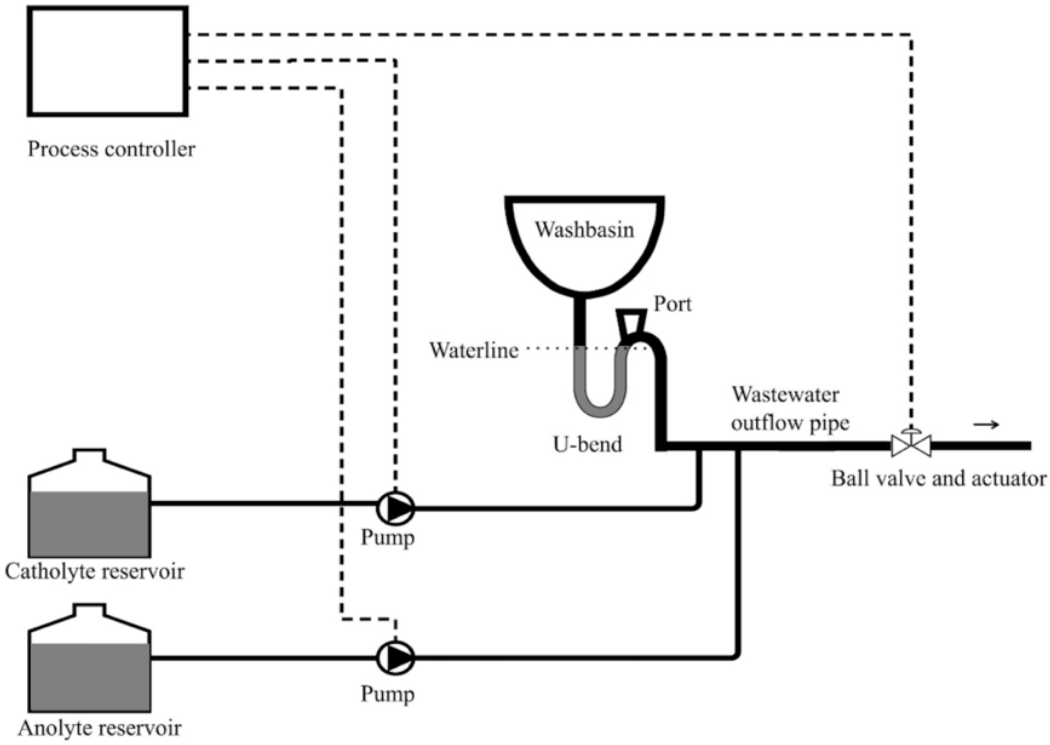

Figure 3.4 General schematic diagram of the second prototype automated washbasin U-bend disinfection system. The programmable process controller initiates disinfection cycles. At the start of each cycle, the actuator closes the valve on the wastewater outflow pipe. After a 20 s delay, catholyte is pumped 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 minutes, the valve opens and the catholyte is voided into the wastewater stream. Then the valve closes and after a 20 second delay anolyte is pumped into until the pipework and U-bend and the cycle proceeds as for catholyte dosing. After 5 minutes, the anolyte is voided into the wastewater stream completing the cycle.

Figure 3.5 Photograph showing the general assembly of the equipment used with the second prototype automated ECA U-bend decontamination system developed in the present study. The system was installed in a first floor staff rest room at DDUH. The process controller is located above the cabinet housing the dosing pumps and ECA reservoirs and is not shown in this picture.

3.1). In contrast, the average bacterial density from the ECA-treated U-bend on CBA, R2A, PA and PAS was 2.1(±4.5), 13.1(±30.9), 0.7(2.8) and 0(±0) CFU/swab, respectively (Table 3.1). For all four media the five-log reduction in bacterial density achieved between the ECA-treated and untreated U-bends was significant (Table 3.1). Figures 3.6 to 3.9 show the relative bacterial densities recovered on a range of microbiological culture media from the ECA-treated and control U-bend between January and April 2016.

3.5.1 Bacterial species recovered from the ECA-treated and control U-bends

The bacterial species identified from different colony types cultured from the test and control U-bends during the pilot and automated disinfection 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.

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

3.5.2 Visual and electron microscope examination of the ECA-treated and control U-bends immediately after the automated U-bend ECA-treatment period

Immediately following the completion of the ECA treatment period of the present study, the U-bends from the ECA-treated and control washbasins were removed and cut in cross section. Direct visual examination of the control U-bend revealed the presence of dense slimy biofilm covering the entire inner surface of the U-bend and not just the areas that would have been filled with water while attached to the washbasin (Figure 3.10). In contrast, the ECA-treated U-bend was remarkably free of biofilm.

Scanning electron microscopy of several sections of each U-bend revealed the presence of dense biofilm in the control U-bend and its virtual absence in the ECA-treated U-bend (Figure 3.11).

Table 3.1 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.

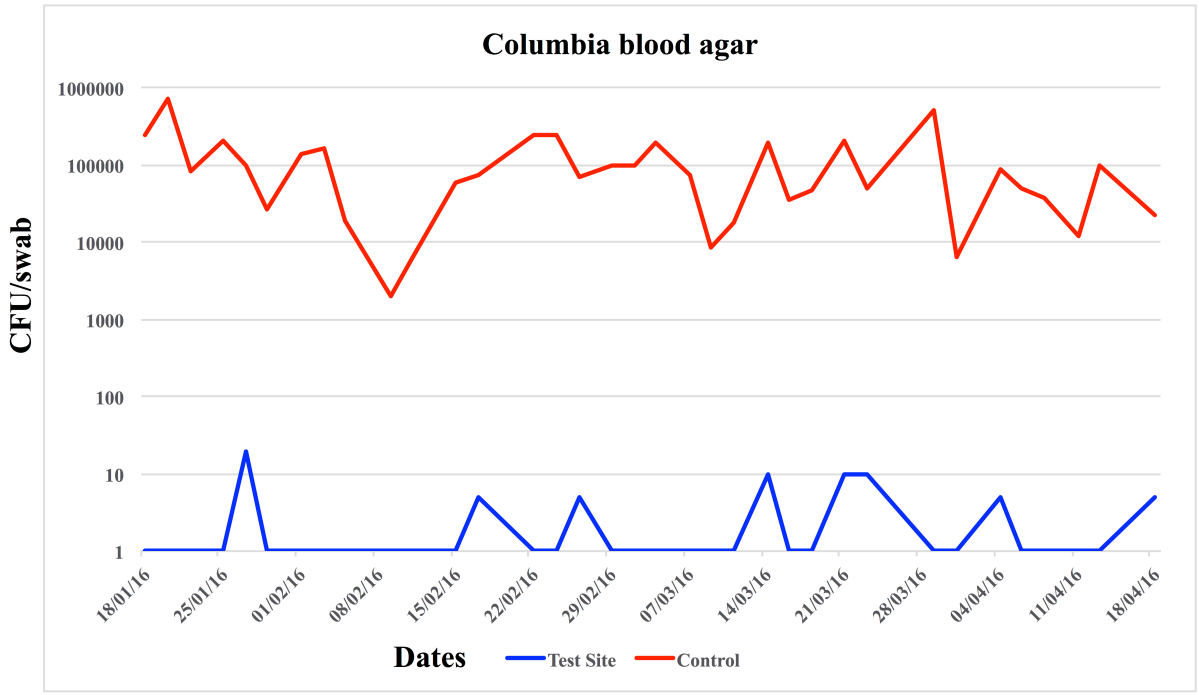

Figure 3.6 Bacterial density recovered on CBA from the ECA-treated washbasin U-bend and the control washbasin U-bend between January and April 2016.

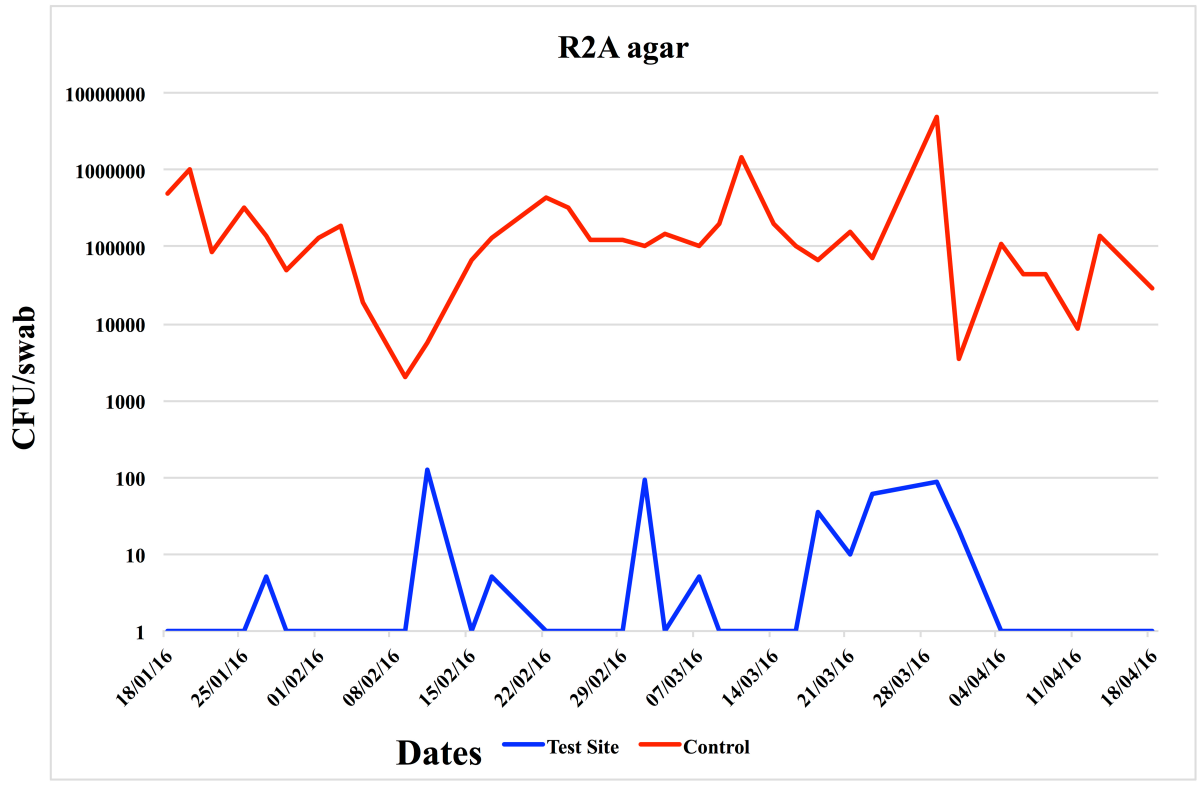

Figure 3.7 Bacterial density recovered on R2A agar from the ECA-treated washbasin U-bend and the control washbasin U-bend between January and April 2016.

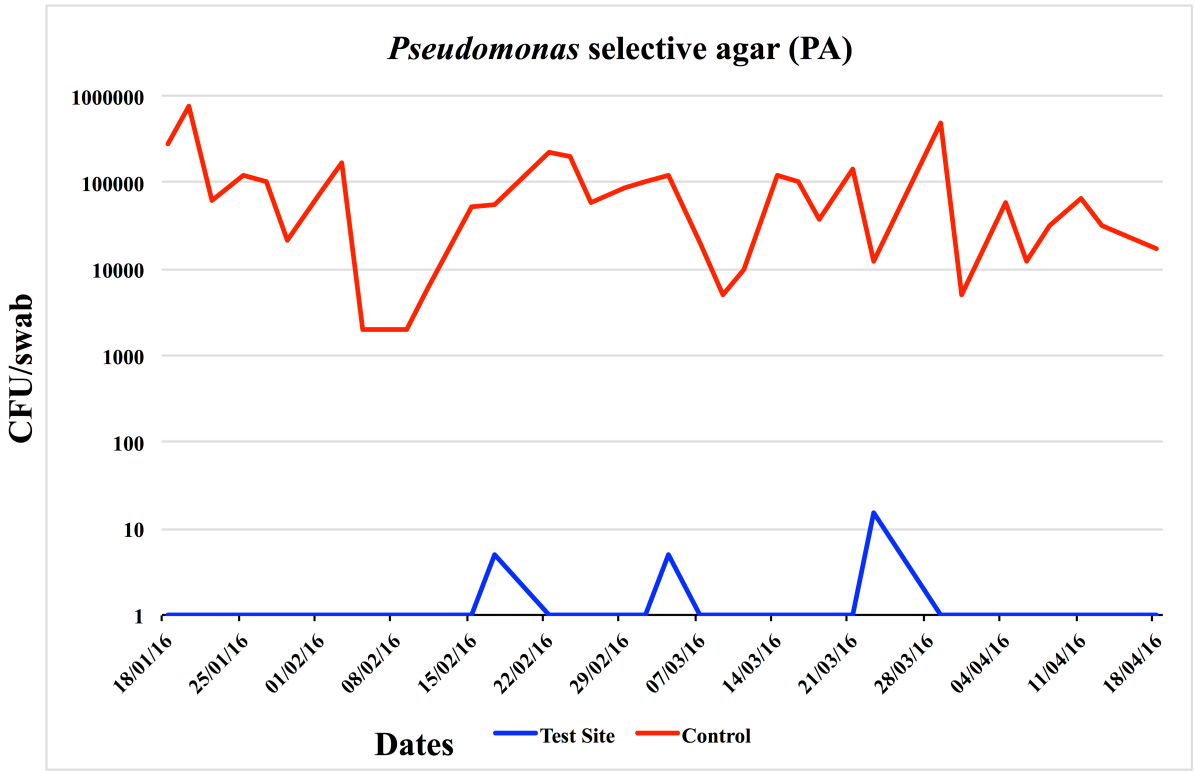

Figure 3.8 Bacterial density recovered on PA medium from the ECA-treated washbasin U-bend and the control washbasin U-bend between January and April 2016.

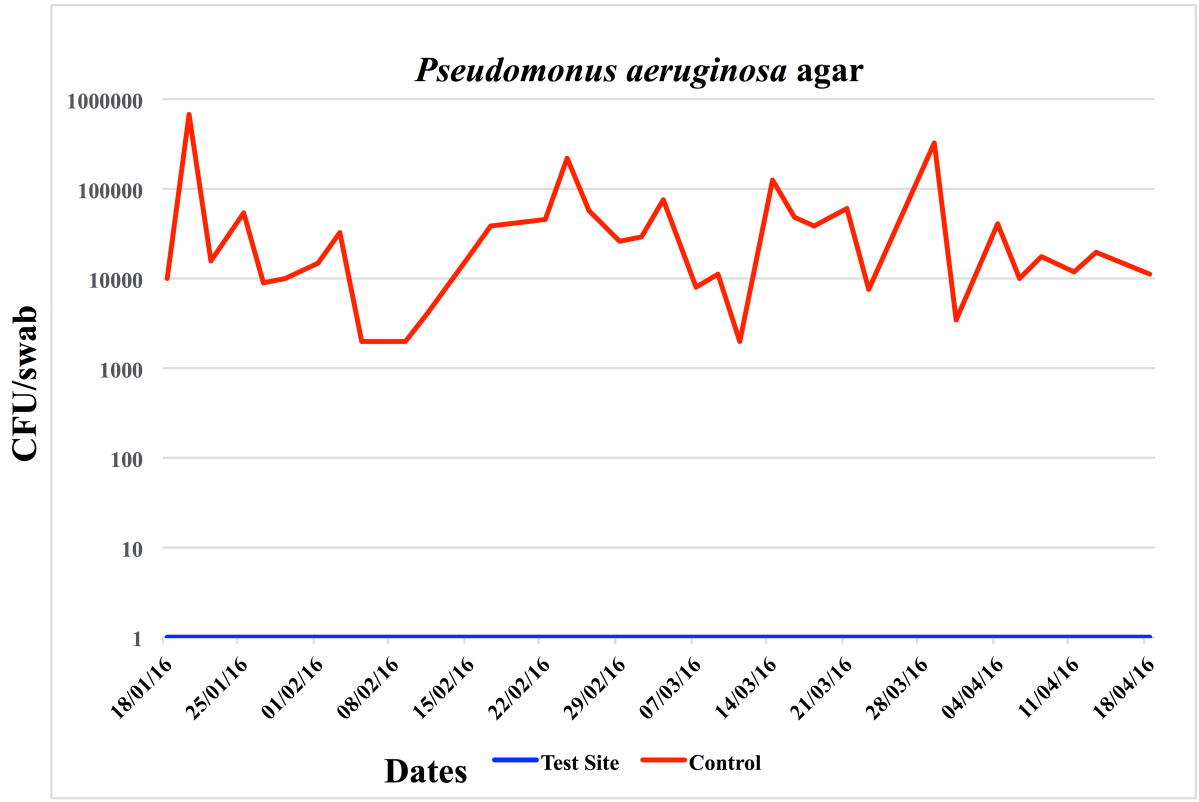

Figure 3.9 Bacterial density recovered on *Pseudomonas aeruginosa* selective agar from the ECA-treated washbasin U-bend and the control washbasin U-bend between January and April 2016.

Figure 3.10 Photograph showing cross-sections of the ECA-treated (left) and control (right) U-bends used during automated washbasin U-bend decontamination. The ECA-treated U-bend was subjected to 35 cycles of treatment with catholyte for 5 min followed by anolyte treatment for 5 min over three months using the second prototype automated U-bend decontamination system developed during the study. ECA treatment cycles were undertaken three times a week on Mondays, Wednesdays and Fridays. The control U-bend was not treated with ECA-solutions. Both U-bends were fitted at the same time to identical washbasins in adjacent bathrooms. The control U-bend is heavily fouled with dense pigmented microbial biofilm. In contrast, the ECA-treated U-bend is virtually free of biofilm. These findings are supported by electron microscopy of internal sections of both U-bends (see Figure 3.11).

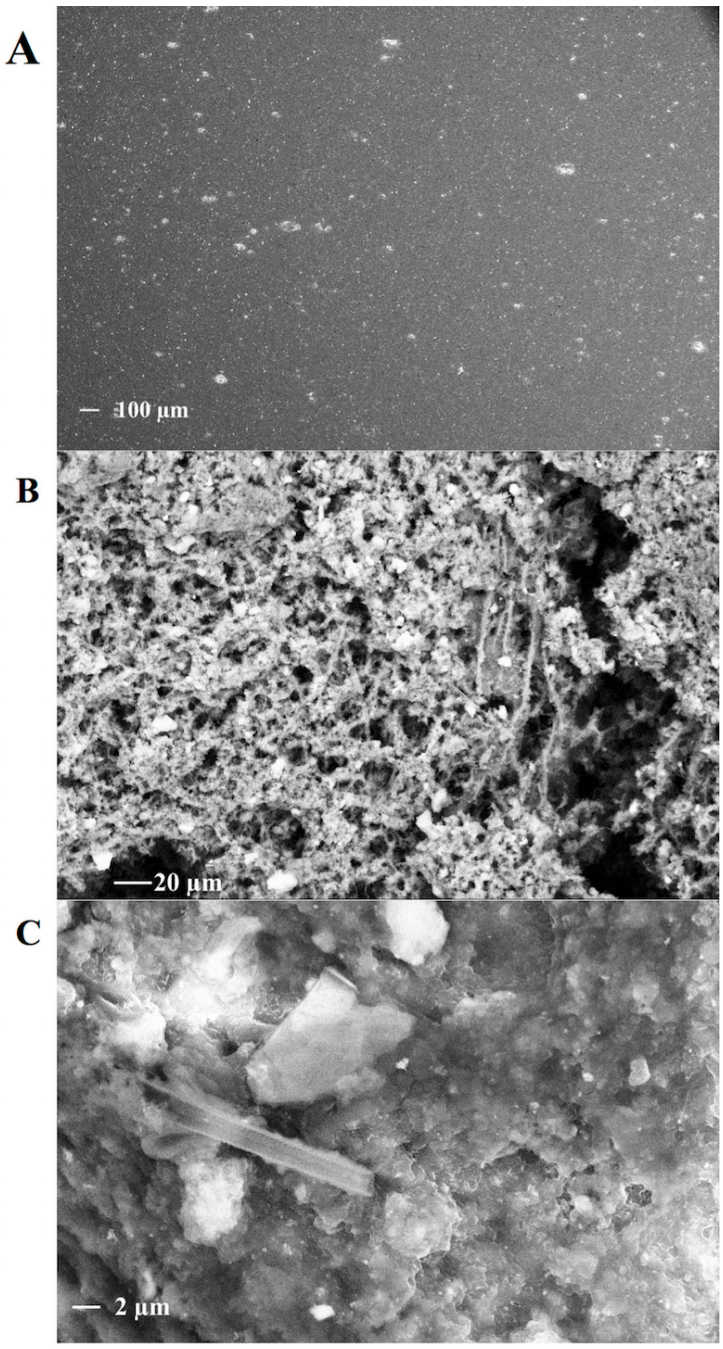

Figure 3.11 Electron micrographs of sections of the internal surfaces of the ECA-treated test U-bend (panel A) following 35 cycles of ECA treatment and a non-ECA-treated control U-bend (panels B and C). The test U-bend was subjected to three weekly treatment cycles (Monday, Wednesday and Friday) with catholyte for five min followed by anolyte for a further five min for a three-month period (35 cycles in total). The ECA-treated U-bend sample is remarkably clean and free of biofilm in contrast to the dense microbial biofilm present in the non ECA-treated control U-bend samples. Size reference markers are shown on each image.

Chapter 4 Discussion

4.1 Discussion

Plumbing networks have formed an integral part of building infrastructure including those of hospitals and other healthcare facilities for many decades. This includes hot and cold water distribution systems, water storage tanks, calorifiers, sinks, hand washbasins, showers, toilets, water filters, faucets, and in the case of healthcare facilities, medical devices supplied with water such as dental chair units and ventilators, amongst many others (Cholley *et al*., 2008; Coleman *et al*., 2009; Coleman *et al*., 2010; Hota *et al*., 2009 Decker and Palmore, 2013; Loveday *et al*., 2014; Blom, 2015; Bloomfield *et al*., 2015; Walker and Moore, 2015; Capelletti and Moraes, 2016). Engineers responsible for mechanical services design in hospitals have, in some cases, over specified the requirement for stored water (Centers for Disease Control and Prevention and American Water Works Association, 2012). Many hospitals and other healthcare facilities have been equipped with water tanks, the capacity of which far exceeds the actual demand for water in these healthcare premises. Providing extra redundancy in terms of water storage leads to underutilisation resulting in water stagnation, which provides suitable conditions for microbial growth. Plumbing networks can be large, intricate with many varied components, which by their very nature are wet, frequently warm, periodically stagnant and prone to contamination with microorganisms and microbial biofilm formation (Coleman *et al*., 2010; Decker and Palmore, 2013; Blom, 2015; Capelletti and Moraes, 2016). Ideally plumbing networks should be zoned where possible and controls put in place to mitigate infection risks. The most critical factors concerning minimising microbial proliferation in all plumbing system involve the eradication of dead legs, adequate thermal controls and reducing opportunities for water stagnation (Health Protection Surveillance Centre, Ireland, 2015). Maintaining cold water supplies below 20°C in large buildings with intricate water distribution networks can be achieved using proprietary cooling systems that engage when the water temperature reaches or gets close to 20°C (Health Protection

Surveillance Centre, Ireland, 2009). Maintaining a constant flow of water within the pipe network is essential to avoid stagnation (Health Protection Surveillance Centre, Ireland, 2015). This is less of an issue in smaller healthcare facilities where the water network is in regular use. For larger buildings, the water distribution system can be designed to ensure flow. Automatic tap control technology can be utilised in critical hospital areas such as ICUs which permits taps to be automatically flushed if they have not been used within a predetermined time period (Health Protection Surveillance Centre, 2015). These measures are more suitable and economically advantageous in new hospital builds. However, risk assessments of critical areas in existing hospital and healthcare facilities can be undertaken to determine the level of modifications required to mitigate infection risks using such new technology. Design engineers for building service control systems have focused primarily on *Legionella* bacterial species responsible for Legionnaire's disease (Heath Protection Surveillance Centre, Ireland, 2009). Much less attention has been paid to microorganisms that are inherently more abundant in plumbing systems, particularly Gram-negative bacterial species such as *S. maltophilia* and especially *P. aeruginosa* (Wang *et al*., 2017). Further consideration should be given to control systems to mitigate all infection risks from plumbing systems.

The chemical and microbiological quality of potable water for human consumption is strictly regulated and much effort has been expended in ensuring potable supplies are safe (Anonymous, 1998). However, the involvement of water distribution networks in the transmission of infectious microorganisms has become increasingly recognised (Decker and Palmore, 2013; Loveday *et al*., 2014; Blom, 2015; Bloomfield *et al*., 2015; Walker and Moore, 2015; Capelletti and Moraes, 2016). Over the past two decades there have been numerous cases of nosocomial infection associated directly or indirectly with washbasin and sink U-bends and drains, a significant proportion of which involved *P. aeruginosa* (Pitmen *et al*., 2001; Hota *et al*., 2009; La Forgia *et al*., 2010; Breathnach *et al*., 2012;

Lowe *et al*., 2012; Starlander and Melhus, 2012; Roux *et al*., 2013; Vergara-López *et al*., 2013; Wolf *et al*., 2014; Leitner *et al*., 2015; Wendel *et al*., 2015; Chapuis *et al*., 2016; Salm *et al*., 2016; Amoureux *et al*., 2017; De Geyter *et al*., 2017).

More recently, the advent of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBLEs) and carbapenemase-producing *Enterobacteriaceae* (CPEs) has further complicated this situation (Lowe *et al*., 2012; Roux *et al*., 2013; Vergara-López *et al*., 2013; Doi *et al*., 2015 Leitner *et al*., 2015; Chapuis *et al*., 2016; Meletis, 2016; Soothill, 2016; White *et al*., 2016; DeGeyter *et al*., 2017; Muzslay *et al*., 2017). ESBLEs confer resistance to penicillins, cephalosporins, and monobactam antibiotics. CPEs are nearly always resistant to carbapenem antibiotics and many other classes of commonly used antimicrobial agents. The management of infections caused by ESBLEs and CPEs poses a significant challenge in clinical practice (Doi *et al*., 2015; Meletis, 2016). There have been a number of reports linking nosocomial infections with these organisms with washbasin and sink drains and U-bends in hospital areas with particularly vulnerable patients including intensive care units and a haematology ward (Breathnach *et al*., 2012; Lowe *et al*., 2012; Starlander and Melhus, 2012; Roux *et al*., 2013;Vergara-López *et al*., 2013; Wolf *et al*., 2014; Leitner *et al*., 2015; Wendel *et al*., 2015; Chapuis *et al*., 2016; Stjärne Aspelund *et al*., 2016; White *et al*., 2016; De Geyter *et al*., 2017;).

Overall, previous efforts at managing contamination risks from washbasin and sink drains and U-bends have focused on treating the contamination itself rather than dealing with the underlying causes. The majority of attempts at managing such contamination have utilised chemical disinfection as a short term measure, but which has been largely ineffective in the long term due to inadequate disinfection, lack of biofilm removal and rapid recontamination (Hota *et al*., 2009; La Forgia *et al*., 2010; Lowe *et al*., 2012; Vergara-López *et al*., 2013; Wolf *et al*., 2014; Fusch *et al*., 2015; Leitner *et al*., 2015; Wendel *et al*., 2015; Stjärne Aspelund *et al*., 2016). A more radical approach has involved

the replacement of sanitary fixtures including washbasins, taps and U-bends in hospital settings, which again has provided a short-term solution, but not in the long term due to rapid recontamination of the new fixtures (Vergara-López *et al*., 2013; Leitner *et al*., 2015; Wendel *et al*., 2015; Stjärne Aspelund *et al*., 2016; De Geyter *et al*., 2017).

The present study adopted a radical new approach to the problem of washbasin drain and U-bend contamination utilising ECA solutions. This study investigated whether ECA solutions could be used to minimise microbial contamination in washbasin U-bends using regular automated treatment and commonly used engineering techniques. The advent of engineering controls using customised programmes such as Fortran and Scada modified to suit a range of widely available PLC's, sometimes called microcontrollers, have significant potential for application in healthcare facilities and can provide very powerful yet affordable real-time quality management tools (Alyami *et al*., 2009). Figures 2.6 and 2.9 in Chapter 2 outline the engineering process diagrams used to map automation of the decontamination process to the Open System Solutions PLC programme. An engineering control process, known as Ladder logic (Lee and Hsu, 2004), was then used to connect each of the individual process components of the decontamination system together through the PLC. The PLC receives a number of inputs from the process, which the ladder logic program uses to calculate the outputs to the components, such as the valves, pumps, etc. The PLC microcontroller has a web server imbedded into the main board which allows human machine interface (HMI) to be created via simple web pages which were accessed via a local area network (LAN) connection to the Lenovo Think Pad used with the system, providing the option to vary some of the parameters as desired. This provided the flexibility to optimise the timing of operation of the actuator and ECA dosing pumps to permit efficient automated treatment of the test washbasin U-bend and wastewater pipework.

Because water stagnation in U-bends can result in particularly dense biofilms, the properties of both ECA solutions generated by electrochemical activation of a dilute salt solution were harnessed for U-bend decontamination 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. This manual operation mimicked the automated system process that was subsequently developed. The ECA 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 from the present study an automated system for U-bend decontamination using ECA solutions was developed using a single test washbasin. The protocol for this was the same as the pilot study except that the entire process was automated (Figure 2.9). Substituting the manual valve on the wastewater outlet with an electronic actuator allowed the ball valve to be opened and closed automatically. The automated mechanism to close the outlet consisted of two parts, a ball valve and an actuator. A ball valve to suit the 4 cm diameter wastewater pipe was sourced (Vexve Termomix D32, Sastamala, Finland) but the torque required to turn the valve was 16 Nm. An actuator capable of delivering the required torque proved difficult to source but one was eventually identified (Joventa 16Nm standard actuator Bratislava, Slovakia) following a visit to the International Trade Show for Sanitisation and Heating in 2015 in Frankfurt, the world's largest sanitary engineering fair. Integrating the actuator and dosing pumps, the latter which replaced the ECA reservoir gravity valves, with the appropriate times and component operating schedule removed the requirement for operator involvement. Modifying the PLC programme from the initial automated prototype using gravity feeding of ECA solutions to the second automated prototype using dosing pumps were carried out

in a short period of time. Adapting and optimising the fill and soak times with the automated system were easily undertaken to achieve the best results during the trials. All contact surfaces were designed to be compatible with ECA solutions; pipes used were manufactured either from uPVC or PE and the pumps selected for use (i.e. Chemical resistant TEKNA EVO TCK 603 pumps) were designed for chemical dosing with seals manufactured from FPM, pump heads manufactured from PVDF and pump diaphragms manufactured from PTFE. Like the pilot study the average bacterial density from the control U-bend during the three month study period was >1 x 10^5 CFU/swab (Table 3.1), whereas microbial contamination of the ECA-treated U-bend was virtually eliminated (Table 3.1). 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 *et al*. (2010) to control an *Acinetobacter baumannii* ICU outbreak involved regularly flushing a gallon of diluted bleach through each sink's wastewater outlet and U-bend. 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. The automated system developed during the present study does not require direct staff involvement in U-bend disinfection and ECA solutions are generated on demand. The results of the pilot study found that a once weekly U-bend disinfection 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 (2013) using bleach to control beta-lactamase-producing-*Enterobacteriaceae* in sink wastewater outlets found that daily disinfection was significantly more effective than weekly disinfection. A recent laboratory study suggested the use of copper pipework in sink wastewater outlets may exhibit higher

antimicrobial activity than commonly used PVC pipework (Soothill, 2016) However, it is unlikely if the antimicrobial effect of copper would be sustained in the long term as copper can develop oxidation layers over time (Wains *et al*., 2011).

Pseudomonas aeruginosa was the most prevalent and abundant bacterial species present in untreated U-bend samples in the present study 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*. (2008). Numerous reports have described outbreaks of nosocomial infection due to *P. aeruginosa* linked directly or indirectly with contaminated washbasin or sink drains, U-bends and taps (Döring *et al*., 1991; Pitmen *et al*., 2001; Cholly *et al*., 2008; Breathnach *et al*., 2012; Walker *et al*., 2014; Davis *et al*., 2015; Fusch *et al*., 2015; Garvey *et al*, 2016b; Tissot *et al*., 2016; Zhou *et al*., 2016). In the present study *P. aeruginosa* was not detected in samples from ECA-treated U-bends. Cholley *et al*. (2008) 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. Studies from this laboratory have previously shown that ECA anolyte is a consistently effective disinfectant for minimising microbial contamination of DUWLs and washbasin output water in the long term (> 2 years). In the present study, the detergent/cleaning properties of catholyte and the disinfectant properties of anolyte were exploited to degrade U-bend biofilm. Neither catholyte nor 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 (Boyle *et al*., 2010). Previous studies with self-disinfecting U-bends used a heating element to heat U-bend wastewater to ≥ 85^{o}C followed by vibration cleaning was found to be effective over a 13-month study period (Fusch *et al*., 2015). 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. The automated system developed during the present study only requires electricity for approximately 12 min per disinfection cycle to activate the pumps and valves.

In DDUH, anolyte solutions have been used for more than 10 years to minimise microbial contamination of water networks (O'Donnell *et al*., 2009; Boyle *et al*., 2010; O'Donnell *et al*., 2011; Boyle *et al*., 2012). Generating these solutions on site provides many advantages. ECA solutions are produced at point of use and therefore hospital storage requirements are kept to a minimum. No harsh chemicals are used reducing the risk of adverse effects following accidental contact. ECA solutions are fragrance free, non-toxic and can be generated to the desired concentration without the need for further dilution (O'Donnell *et al*., 2009; Boyle *et al*., 2010). There are no toxic waste streams and no special requirements for disposal as ECA solutions are environmentally friendly and inactivated readily following discharge in wastewater (O'Donnell *et al*., 2009; Boyle *et al*., 2010). The prototype automated requires minimal human intervention saving valuable time for both facilities and clinical staff. Sanitary pipework such as U-bends, waste pipes and associated fittings manufactured from materials such as unplasticised polyvinyl chloride (uPVC) and polyethylene are not damaged following prolonged contact with ECA solutions (see Appendix 1). Unplasticised. PVC is totally compatible with anolyte (1000 ppm at pH 7 to 8) and catholyte (at pH 11-13.5). Polypropylene is compatible with anolyte (1000 ppm between pH 7 to pH 8) but catholyte can cause minor damage at pH 11-13.5. In tests on the manual system the catholyte was diluted at a 1:10 ratio with potable water and a 1:5 ratio with potable water in the automatic system. No signs of fatigue or wear were noted in the U-bends or waste pipes following successive rounds of ECA decontamination.

The most significant risks of infection from clinical washbasins in hospitals occur in areas housing vulnerable patients, such as burns units, paediatric wards, ICU's, geriatric wards, haematology wards, cystic fibrosis wards and oncology wards. Adopting this

technology for use in these areas will present some logistical challenges for both engineers and microbiologists. However, the results of this study demonstrate that active collaboration between microbiologists and engineers can yield effective solutions to the problem of long-standing contamination reservoirs frequently responsible for nosocomial infections.

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

Chapter 5 General Discussion

5.1 Future developments

5.1.1 Gravity fed automated U-bend decontamination

The development and testing of the initial prototype automated U-bend decontamination system relied on delivery of ECA solutions from reservoirs into the test washbasin U-bend and associated wastewater pipework by gravity feed. Initial results were promising when the ECA reservoirs mounted on the wall above the washbasin were full and generated sufficient pressure to gravity feed ECA solutions into the U-bend and pipework so that they were filled completely. However, when the volume of ECA solutions in the reservoirs reduced following initial rounds of U-bend treatment, delivery of sufficient ECA solutions to consistently fill the U-bend and associated pipework became problematic. The obvious cause of this problem was reduced pressure as the volume of solutions in the reservoirs reduced. Full ECA reservoirs always resulted in effective automated filling of the test U-bend and associated wastewater pipework. As the ECA reservoirs began to empty following rounds of U-bend treatment, complete filling of the test washbasin U-bend and pipework was inconsistent. For this reason, delivery of ECA solutions by gravity feed in the present study was abandoned and pumps were used instead. However, it is important to highlight that the ECA reservoirs used for gravity feed were placed 1.5 m above the test washbasin. Significantly increasing the height of the reservoirs above the washbasin should overcome the issue of insufficient pressure encountered in the present study. This could be achieved by locating the ECA reservoirs in the attic space two floors and several metres above the rest room used in the present study. The option to provide ECA solutions by gravity feed would negate the requirement to use electric chemical-resistant pumps and is a less expensive option. However, this option is offset by potential logistical difficulties in regularly refilling ECA reservoirs in an attic space due to accessibility and health and safety considerations. Nonetheless, in a new build situation, gravity feeding ECA solutions would probably be a viable alternative to automated ECA dosing using pumps.

5.1.2 Simultaneous automated decontamination of multiple washbasin U-bends

The results of the present study demonstrated that it is possible to effectively and consistently decontaminate washbasin U-bends with ECA solutions using an automated system such as that developed in the present study. The obvious extension of this work would be to further develop the system for simultaneous large-scale application to multiple washbasin U-bends.

Ideally, upscaling this project and integrating it into a live clinical environment would be carried out during a refurbishment or redevelopment of an existing clinical area or a new build clinical area. During the course of this study, plans for the refurbishment of the DDUH Accident and Emergency (A&E) Department were proposed. This development provides a unique opportunity to integrate and test a large-scale automated washbasin U-bend decontamination system serving multiple washbasins at DDUH. Plans to develop an automated large-scale washbasin U-bend decontamination for the DDUH A&E Department are in progress. The location of the DDUH A&E Department is ideal as it is situated directly above the hospital's basement equipment plant room, which houses the hospital's ECA generators and provides easy access to wastewater pipes servicing washbasins in the A&E Department. The proposed large-scale system will use pumps to deliver ECA solutions to washbasin U-bends as the equipment plant room is in a basement area. The refurbished A&E Department will be equipped with 12 hospital pattern washbasins and the wastewater pipes from each will connect to a common 10 cm-diameter wastewater main directly below the A&E Department in the basement plantroom. It will be critical to ensure that all washbasins are installed at precisely the same level above the A&E floor so that the ECA solutions reach the same level within each washbasin during automated filling. The capacity of the collective washbasin U-bends and associated wastewater pipework will be approximately 250 L, which will require larger chemical-resistant dosing pumps and ECA reservoirs than used with the prototype system to

facilitate complete filling of the system with ECA solutions in a reasonable timeframe (i.e. 5-10 min) to a level 50 mm above the drain outlet of each washbasin. A larger ball valve and actuator will also be required to seal the main 10 cm diameter wastewater outlet pipe. ECA solution-resistant wastewater pipes made from uPVC will be used with the large-scale system and similar polypropylene U-bends with inspection ports as used with the prototype system will be fitted to each washbasin. U-bends and pipes for each washbasin will be concealed behind a panel with a small access hatch to facilitate access to services and for U-bend sampling. All pipe joints will be welded to minimise potential leaks. The operation of the large-scale system will be managed by a PLC controller that will be programmed following empirical determination of the exact times required for operation of individual components of the automated process such as ball valve closing and pump operation.

Testing of the efficacy of the large scale automated system will proceed using a similar approach to that used with the prototype system described in the present study. The frequency of ECA treatment necessary to minimise microbial contamination of the washbasin U-bends will be determined empirically following microbiological sampling of each U-bend. Figure 5.1 outlines the general arrangements for the proposed large-scale U-bend-decontamination system using automated treatment with ECA solutions.

5.1.3 Adaptation of automated ECA decontamination for other applications

The results of this study demonstrate that automated ECA treatment of U-bends can effectively manage contamination risks from washbasins. The use of catholyte and anolyte in the prototype system has been proven to achieve effective control of washbasin U-bend contamination suitable for a clinical environment. Data to date indicate that large-scale automated ECA treatment systems will be equally effective. It is highly likely that the ECA decontamination approach developed in this study can be adapted for a variety of other clinical and industrial applications.

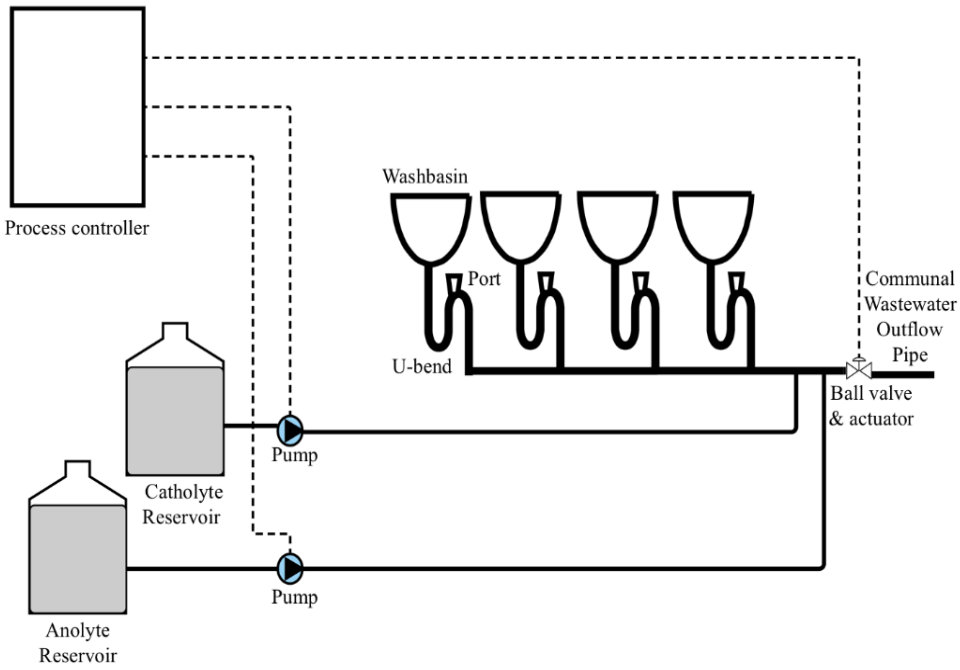

Figure 5.1 General schematic diagram of the proposed large scale U-bend-decontamination system using automated treatment with ECA solutions. Similar to the single washbasin system the programmable process controller initiates disinfection cycles. At the start of each cycle, the actuator will close the valve on the wastewater outflow pipe. After a short delay, the catholyte will be pumped, using a larger capacity dosing pump, into the pipework below the washbasin U-bend until the pipework and U-bend are completely filled to a level a few cm above each of the washbasin wastewater outlets. After 5-10 min, the valve will open and the catholyte will be discharged to waste. Then the outflow valve will close and after a further short delay the anolyte will be pumped into the pipework and U-bend and the cycle will proceed similar to the catholyte dosing. After 5-10 min, the anolyte is discharged to waste completing the cycle.

5.1.3.1 Showers

Showers are well recognised as vehicles for transmission of Legionnaire's disease *via* contaminated aerosols, including in the hospital setting (Health Protection Surveillance Centre, Ireland, 2009). However, showers have also been linked with transmission of other bacteria. Shower wastewater outlets called traps carry out a similar function to U-bends on washbasin and sink drain wastewater outlets and have been implicated in nosocomial infections and outbreaks of *P. aeruginosa* (Breathnach *et al*., 2012; Quick *et al*., 2014; Blom, 2015; Tissot *et al*., 2016). The automated ECA decontamination developed in the present study could be adapted to treat shower drains. The approach would be similar to that in the prototype decontamination system developed in the present study with some design changes. Shower traps tend to be shallow as they are designed to fit under a shower tray and under the floors of existing buildings where there are usually space restrictions. The trap on a shower tray carries out the same function as a U-bend on a washbasin or sink but the location of the trap below a shower tray and under floorboards usually renders them inaccessible. Testing a system using an existing shower outlet would be more challenging until the system is proven, after which the valve fitted on the wastewater pipework to retain ECA solutions during decontamination could be fitted outside the limits of the shower tray for accessibility.

Shower hoses are also known to harbour bacteria including *Legionella*, *P. aeruginosa* and microbial biofilm, as they do not empty fully after use and thus provide an ideal environment for microbial proliferation. It should be possible to adapt the prototype system developed in this study to incorporate the shower hose into a closed loop system for decontamination with ECA solutions. This approach would be ideal to use in conjunction with activated shower mixer taps that can be programmed to flush at regular intervals or when the shower has not been used for a predetermined period of time.

5.1.3.2 Healthcare

The application of ECA solutions to decontaminate shower traps and hoses would be of most benefit in minimising contamination reservoirs and in minimising infection risks in hospital critical care areas including ICUs, haematology wards and cystic fibrosis wards, amongst others. However, this approach may also be beneficial to use in the homes of cystic fibrosis patients. This patient group is particularly vulnerable to pulmonary infections with *P. aeruginosa* and related bacterial species (Moradali *et al*., 2017). Adapting the prototype ECA decontamination system to a self-contained unit which could include the actuator and valve to fit onto an existing 4 cm-diameter wastewater pipe in a domestic setting could be considered. Adapting the control PLC used in the prototype system for use with widely available mini microprocessor units such as the Raspberry Pi or similar could make this an economically viable solution. A Raspberry Pi is a tiny and affordable computer, which is about the size of an iPhone (O'Briain, 2014). Replacing the PLC with a Raspberry Pi in a domestic environment with minimal inputs/outputs would make manufacturing a domestic unit economical viable.

Heater-cooler devices are used during surgical procedures involving heart and lungs to warm or cool a patient. They are intended to keep the circulating blood and organs at a specific temperature best suited for the type of surgery. In recent years there have been several reports of *Mycobacterium chimaera* infections in patients following cardiac surgery associated with contaminated heater-cooler units, which were linked with exposure to contaminated aerosols generated by the units (Sax *et al*., 2015; Garvey *et al*., 2016a). The CDC (Centers for Disease Control and Prevention) has advised healthcare providers and patients about the potential risk of infection from certain devices used during open-heart surgery. (https://www.cdc.gov/media/releases/2016/p1013-contaminated-devices-.html). It should be readily straightforward to adapt the automated ECA decontamination system

developed during this study to decontaminate heater-cooler water tubes to minimise risks of infection.

5.1.3.3 Industrial and other applications

Current methods used for cleaning pipelines between production cycles at bottling plants utilise *clean in place* (CIP) systems, which involve removing residues and odours from pipelines at product manufacture changeovers. CIP is principally concerned with soil removal: soil being anything that should not be present in a clean vessel. It can be visible (scale, foreign bodies,) or invisible in the form of microorganisms. The time needed to remove soil is at least 15 min using a suitable chemical at temperatures above 50°C. Commonly used chemicals for soil removal include caustic soda, phosphoric and nitric acids, sodium hypochlorite and peracetic acid. Caustic soda is an alkali typically used at 0.5% - 2% weight/volume. Phosphoric and nitric acids are used in detergent formulations for scale removal, often at lower temperatures than caustic soda. These acids must be used with care as they can corrode valve and pump seals. They are often used in dairies for one week in every 6 weeks to remove milk scale, and can be used after commissioning to remove installation debris (http://www.process-worldwide.com/what-is-cleaning-in-place-and-how-does-it-work-a-320588/). Beverage industry giants such as Pepsi and Coca Cola use CIP systems at their bottling plants using ECA solutions to save time and money by reducing costs, increasing efficiency and therefore valuable production time. The application of ECA solutions could be particularly useful in other industries where bottling is carried out such as milk production, soft drink production, soup production etc. http://www.miox.com/documents/Article_Bottling_a_Winning_Clean-in-Place_Solution.pdf).

Enclosed water systems are used extensively in the poultry production industry. Water is provided using long drinker lines fitted with nipples that dispense water. Birds obtain water on demand by pushing a pin on the bottom of the drinker line. These drinker

lines are also used to deliver vaccines and medications. As with all water networks, microbial contamination can occur, which can affect the health and performance of poultry flocks. Microbial contamination above acceptable levels in drinking water can directly affect poultry health and performance (Maharjan *et al*., 2016). Minimising microbial contamination of the water supply and drinker lines is essential. The importance of this issue is reflected by the implementation of more stringent controls on the use of antibiotics in food animals. The application of automated ECA solution technology could be readily adapted to provide a safe and efficient means of ensuring safe drinking water for poultry.

Water is used extensively in the horticultural industry in large green houses to provide water and feed to plants. This water is regularly aerosolised and is a potential source of *Legionella* bacteria. Automated ECA decontamination approaches could be used in the treatment of plants in greenhouses against bacterial infections and insect infestations. (http://www.vbinstitute.org/technologies/#food). Similarly at car wash stations or indeed any situation where water is aerosolised from equipment systems could benefit from the use of automated ECA decontamination technology.

Meat processing plants carry out extensive cleaning regimes particularly in slaughter halls and on boning tables where strict adherence to industry standards is required. Meat is prepared and packaged on stainless steel tables using chopping boards and the off-cuts are discarded through chutes. Meat is then distributed to different areas of the boning hall depending on the cut of meat. Physical cleaning with pressurised water may stir up dirt or produce contaminated water droplets (aerosol), which could contaminate meat present in these areas of production. Chemical cleaning/disinfection may produce toxic residues when in contact with remaining meat or meat products. Detergents currently in use could also contain additional cleaning agents such as chlorine, silicate or phosphate. Using electrochemical activated (ECA) solutions in the treatment of food raw materials at food plants, for example at beet processing factories

(http://www.vbinstitute.org/technologies/#food) could be a viable and environmentally friendly alternative.

5.1.4 Overview

Automated ECA decontamination can be used to provide a versatile approach to managing a range of infection risks in healthcare facilities. This technology has been used successfully for over a decade at DDUH to minimise infection risks from DUWLs, washbasin and sink hot and cold output water and taps. The results of the present study demonstrate that this technology can also be used effectively to minimise infection risks from washbasin U-bends and drains, which have been previously associated with numerous nosocomial outbreaks. Expanding the range of applications for automated ECA decontamination in healthcare makes good economic sense as the same ECA generator can be used to provide solutions for a wide variety of applications. The proven efficacy of ECA solutions at minimising infection risks and the fact that they provide a viable, effective and environmentally friendly alternative to harsh chemical disinfectant agents make them particularly attractive for widespread use in healthcare and in a variety of industrial and other applications.

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Appendix 1

Appendix I: Materials compatible with anolyte and catholyte solutions

Material	Anolyte 1000 ppm	Catholyte 13.5 pH	Anolyte 40 ppm	Catholtye 11.5 pH
Polyethlyene (PE)	A	A-B	A	A
Polytetrafluoroethylene (PTFE)	A	A	A	A
Fluoro-rubber (FPM)	A	A	A	A
Polyvinyl chloride (PVC)	A	A	A	A
Polyvinylidene fluoride (PVDF)	A	A	A	A
Latex	C	D	C	C
Silicone	D	C	D	D
Stainless steel	D	C	A	D
Aluminium	D	D	D	D
Copper and brass	D	D	D	D
Iron	D	D	D	D

Category A materials are unaffected by contact with ECA solutions

Category B materials are either unaffected or moderately affected by contact with ECA solutions

Category C materials are affected by contact with ECA solutions resulting in cracking, discoloration, softening or swelling

Category D materials are no recommended for continuous use with ECA solutions

(Data provided courtesy of Thomas Johnson, Chief manager/founder Qlean Tech Enterprises, LLC, 1408 Northland Dr. #406 Mendota Heights, MN 55120, USA: tom@qleantech.com)