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

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SUMMARY

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

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

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

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

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

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Introduction

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

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

A variety of approaches to U-bend decontamination have been investigated with varying success, most of which involve disruption to service and have financial implications, including the replacement of fixtures and/or associated pipework [2,6,10]. Replacement is ineffective in the long term as new washbasins and pipework rapidly become recolonized with micro-organisms. Disinfectants such as bleach may have diminished efficacy against dense biofilms, temporarily reducing bioburden but necessitating regular application [2,3,10]. Another approach involves thermal disinfection and vibrational cleaning of U-bends, but is not in widespread use [12].

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

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

Methods

Anolyte and catholyte

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

Test and control washbasins

Ten new ceramic hospital pattern washbasins with an offset drain outlet in the back wall of the basin (Armitage Shanks, Stoke on Trent, UK) were installed at the Accident & Emergency Department of the Dublin Dental University Hospital (DDUH) for ECA decontamination studies. Six identical washbasins located in different DDUH clinics were used as controls. Washbasins were used solely for handwashing. Tork Extra Mild Liquid Soap (SCA Hygiene Products Ltd, Dunstable, UK) was used for handwashing at all washbasins. Cold water supplied to test and control washbasin taps was provided from a 15,000-L tank supplied with potable quality mains water. This tank also supplied the calorifier, which provided hot water to all the washbasin taps. Automatic temperature recording was fitted on the out and return legs of the hot water network. Washbasin taps are fitted with a thermostatic mixing valve and provided output water at an average temperature of 38°C. Hot and cold water supplied to washbasins at DDUH has been treated with residual anolyte (2.5 ppm) for several years. Previous studies over 54 weeks showed average bacterial densities in hot and cold tap water of 1 [standard deviation (SD) 4] and 2 (SD 4) colony-forming units (cfu)/mL, respectively [14]. All washbasins were in frequent daily use from Monday to Friday. Three months prior to the study, washbasins were equipped with new

polypropylene U-bends (McAlpine Plumbing Products, Glasgow, UK) with two access ports (Figure 1).

Design of automated ECA treatment system for U-bends

A large-scale system was developed to decontaminate 10 washbasin U-bends, drains and proximal wastewater pipework simultaneously (Figure 2). A vertical wastewater pipe below each U-bend was connected to a horizontal common wastewater collection pipe. The pipes and fittings were made of polyvinylchloride (PVC) or acrylonitrile-butadiene-styrene (ABS), both compatible with long-term exposure to anolyte and catholyte. All pipe connections apart from U-bends were chemically welded to minimize the potential for leaks. ECA reservoirs were manufactured from ultraviolet-stabilized



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

linear polyethylene designed for chemical storage. Each reservoir supplied a dosing pump (Grundfos, Bjerringbro, Denmark) connected by 25-mm ABS pipework to the common wastewater pipe (Figure 2).

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

Automated ECA decontamination cycles

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

Microbiological culture

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

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

Identification of bacterial isolates

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

Electron microscopy

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

Statistical analysis

Statistical analyses were performed using GraphPad Prism v.5 (GraphPad Software, San Diego, CA, USA). Statistical significance was determined using unpaired, two-tailed Student's t-test with 95% confidence intervals. Statistical significance of more than two sets of data was determined using one-way analysis of variance.

Results

Automated U-bend decontamination

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

Table I

Agar medium	U-bend	Average bacterial counts in cfu/swab from ECA-treated (N = 62 cycles, 620 swabs) and control $(N = 372$ swabs) U-bends	SD	Range of bacterial counts in cfu/swab	<i>P</i> -value
СВА	Treated Untreated	73.4 2×10^5	258.2 4 × 10 ⁵	$0-4.6 imes 10^{3}\ 0-4 imes 10^{6}$	<0.0001
R2A	Treated Untreated	122.5 3.3×10^{5}	371.3 1.1 × 10 ⁶	$\begin{array}{c} 0{-}5.8\times10^{3}\\ 0{-}1.8\times10^{7} \end{array}$	<0.0001
PAS	Treated Untreated	15.3 2.7 \times 10 ⁴ Average bacterial counts in cfu/swab 24 h after ECA treatment (<i>N</i> = 42 cycles, 420 swabs) and control (<i>N</i> = 252 swabs) U-bends ^a	184.5 1.2 × 10 ⁵	$\begin{array}{c} 0{-}3.4\times10^{3}\\ 0{-}1.4\times10^{6} \end{array}$	<0.0001
CBA	Treated ^a Untreated	53.2 2.1 × 10 ⁵	127.6 4.3 × 10⁵	$0-1 \times 10^{3}$ 500-3.2 $\times 10^{6}$	<0.0001
R2A	Treated ^a Untreated	91.7 2.9 × 10⁵	277.6 6.1 × 10 ⁵	$0-3.5 \times 10^{3}$ $1.3 \times 10^{3}-5 \times 10^{6}$	<0.0001
PAS	Treated ^a Untreated	15.6 2.6 × 10 ⁴	119 1.1 × 10⁵	$\begin{array}{c} 0{-}1.7\times10^{3}\\ 0{-}1.4\times10^{6} \end{array}$	<0.0001

Average quantitative bacterial counts from 10 washbasin U-bends subjected to automated treatment with electrochemically activated solutions (ECA) and the corresponding counts from six untreated U-bends

CBA, Columbia blood agar; R2A, Reasoner's 2A agar; PAS, *Pseudomonas aeruginosa* selective agar; SD, standard deviation; cfu, colony-forming units. ^a The average bacterial counts in cfu/swab were determined for the 10 ECA-treated U-bends and the six untreated U-bends 24 h after treatment for 42 of 62 ECA treatment cycles.

All 10 test washbasins were exposed to three weekly decontamination cycles (Monday, Wednesday and Friday) over five months (62 cycles), which was almost double the number of cycles assessed in the previous proof of concept study. Six additional washbasins located elsewhere in DDUH were used as controls. Swab samples were taken from the internal surfaces of the U-bends, and semi-quantitative bacterial counts were determined on CBA, R2A and PAS. The average bacterial density from the six untreated U-bends during the study on CBA, R2A and PAS was 2×10^5 (SD 4×10^5), 3.3×10^5 (SD 1.1×10^6) and 2.7×10^4 (SD 1.2×10^5) cfu/swab, respectively (Table I). For the 10 ECA-treated U-bends over 62 cycles, the average bacterial density on CBA, R2A and PAS was 73.4 (SD 258.2), 122.5 (SD 371.3) and 15.3 (SD 184.5) cfu/swab, respectively (Table I). The average reduction in viable counts from ECAtreated U-bends was $>3 \log$ or a 99.9% reduction. Reductions in average bacterial counts from treated U-bends on all media relative to the counts from control U-bends were highly significant (P<0.0001) (Table I). There was no significant difference in average bacterial counts on all media between the 10 individual treated U-bends over the study period (P>0.4). Additional U-bend samples taken from all 10 treated U-bends 24 h after treatment for 42 of 62 decontamination cycles showed no significant increase (P>0.1) in average bacterial counts on all media (Table I).

Bacterial species identified from U-bends

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

Biofilm on ECA-treated and control U-bends

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

Biofilm on washbasin drain outlet surfaces

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

Adverse effects on washbasin wastewater network

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

Discussion

Proof of concept for effective and consistent decontamination of washbasin U-bends by automated sequential treatment with catholyte followed by anolyte was demonstrated in a previous study using a single domestic pattern washbasin located in a hospital washroom [8]. The present study developed a novel automated ECA treatment system to decontaminate 10 hospital pattern washbasin U-bends, drain outlets and proximal wastewater pipes simultaneously in a busy hospital department. The results of the study demonstrate that the large-scale system has a comparable decontamination efficacy to the pilot system, as both resulted in a >3 log reduction in bacterial counts in treated U-bends relative to controls (P < 0.0001). However, with the large system, $>3 \log$ reductions were achieved simultaneously in 10 separate U-bends in a busy hospital clinic, demonstrating that this approach has good potential for application in hospital departments and wards equipped with multiple washbasins. In the pilot study, P. aeruginosa was not recovered from the ECA-treated U-bend. The finding of low densities of P. aeruginosa in some ECAtreated U-bends within the larger system is not surprising because of its larger and more extensive network of pipes servicing 10 washbasins. All control and ECA-treated U-bends were positive for P. aeruginosa at some point during the study, indicating that it is endemic within the wastewater network. Similarly, Cholley et al. sampled 28 U-bends over eight weeks and found that all were colonized at least once by P. aeruginosa [1]. In the present study, and in the pilot study, bacterial counts recovered immediately after ECA treatment and 24 h later were similar on all media tested, which demonstrated that biofilm within the pipework did not recover rapidly from ECA treatment [8]. A limitation to the present study is that the authors did not demonstrate that this approach would help to control an actual hospital outbreak associated with contaminated U-bends.

A variety of Gram-negative bacterial species other than *P. aeruginosa* were identified in ECA-treated and control U-bends (Table A, see online supplementary material).

However, a greater range of Gram-positive species was identified from treated U-bends due to the recovery of several staphylococcal species that were not identified in the controls (Table A, see online supplementary material). Staphylococci are common skin commensals that inevitably get transferred into U-bends during handwashing. The recovery of staphylococci from treated U-bends, albeit in low numbers, could be due to their presence being masked by high densities of Gramnegative bacteria within the control samples.

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

The majority of previous approaches to control hospital outbreaks linked to contaminated U-bends and drains have involved pouring chemicals down the drain outlets and/or replacing the washbasin and/or associated pipework [2,3,6,10]. Vergara-López et al. installed manual shut-off valves into sink drainage pipes, followed by 30-min treatment with a guaternary ammonium compound and subsequent flushing with hot water to control a Klebsiella oxytoca hospital outbreak [5]. A number of valves had to be manually operated prior to manual addition of the disinfectant, which may lead to air being trapped in the pipework, shielding some areas from disinfection. In contrast, the ECA decontamination system developed and tested in this study is automated and backfills the pipework from below each U-bend, reducing the likelihood of air being trapped. A recent study showed that sink-to-sink transmission can occur via a common wastewater pipe [9]. The approach used in this study minimizes opportunities for transmission of organisms between U-bends connected by common wastewater pipework, as the system decontaminates drains, U-bends and pipework.

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

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Conflict of interest statement None declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jhin.2018.01.012.

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