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# Hospital Sinks and Drains as a Source of Antimicrobial Resistant Organisms: Studies to Investigate Colonisation, Dispersal and Decontamination

## Efficacy of chemical disinfectants and impact of active agent(s) and formulation

Biosafety, Air and Water Microbiology Group, PHE Porton

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# About Biosafety, Air and Water Microbiology Group

The Biosafety, Air and Water Microbiology Group at Porton Down has been carrying out independent evaluations of infection control interventions in laboratories, health care, containment, workplace and domestic settings for over twenty years. Our expertise is in air and water microbiology applied to nosocomial, pharmaceutical and containment situations. We have developed and offer standard techniques for the determination of the efficacy of filters and air disinfection units, the performance of safety cabinets, sealed centrifuges rotors and air samplers. We are also able to assess liquid and gaseous disinfectants and the microbial air quality of healthcare facilities, workplaces and other environments.

The Biosafety, Air and Water Microbiology Group provides specialist bespoke research, testing and evaluation services for commercial customers that delivers independent analysis and reports. However, as a public sector body we are not able to endorse any particular products or recommend them for use by the NHS or others.

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## Executive summary

The increasing use of broad-spectrum antimicrobials has led to a significant increase in multidrug resistance and the emergence of carbapenemase producing Enterobacterales (CPE) is a major public health concern. CPE are resistant to ß-lactam antibiotics and are also frequently resistant to other classes of antimicrobials. Clinical infections are difficult to treat and are associated with high mortality. Acquisition of CPE primarily occurs among hospitalised patients and patient-associated risk factors have been identified. However, there is increasing evidence linking infections to an environmental source; specifically, hospital sinks, waste traps and/or drains.

The stagnant water within a waste trap facilitates the formation of biofilm comprising many millions of microorganisms including those that are resistant to antibiotics. Use of the sink and/or tap can dislodge and disperse these bacteria onto surrounding surfaces and pose a risk for onward transmission. The eradication of biofilms from hospital drains is difficult and there is a lack of guidance on how to proceed should CPE (or other resistant organisms) be recovered from sinks and/or wastewater plumbing. This study utilised a unique model system to assess the efficacy of different chemical disinfectants when used to treat sinks known to be colonised with antimicrobial resistant Gram-negative organisms.

Chlorine-based and peracetic acid (PAA)-based disinfectants, applied as either a liquid or a foam, were used to treat both base-draining and rear-draining sinks. The number of Gram-negative bacteria present within the waste trap water was monitored over time and the impact of disinfection on bacterial dispersal assessed. Immediately following treatment with either of the liquid disinfectants, the concentration of planktonic bacteria within the sink waste trap fell to below detectable levels (i.e. disinfection achieved at least a 4-log reduction in culturable Gram-negative bacteria) and no bacteria were dispersed to surrounding surfaces. However, neither disinfectant eliminated the colonising biofilm and bacterial levels (and bacterial dispersal) returned to baseline 48 - 72 hours later. The foam-based products were less effective.

Ensuring a sink is frequently and appropriately used (i.e. is not used to discard liquid waste) may help prevent biofilm formation, enhance chemical disinfection and allow for longer-term effect. However, results from this study would suggest that chemical disinfectants (if effective) are a short-term solution and without frequent (e.g. daily) application should not be relied upon to reduce contamination within a sink, prevent bacterial dispersal or reduce the risk of onward transmission.

## Introduction

Hand hygiene is central to national and international campaigns to reduce healthcare-associated infections and encouraging healthcare staff to regularly and effectively decontaminate their hands is a key focus of infection prevention and control in hospitals. Easily accessible sinks can encourage staff to wash their hands. However, easy access to sinks also provides ample opportunity for staff (patients and visitors) to use them inappropriately.

The inappropriate discard of waste material (e.g. patient secretions; body fluids) can directly introduce enteric pathogens, including those that are resistant to antibiotics, to the wastewater system. Foods, paper and plastics, if dropped into the sink, can be washed down the drain and retained within the waste trap. The disposal of debris and liquid wastes (including sterile IV fluids and beverages) will also introduce nutrients to the wastewater system which, in turn, will facilitate the survival and growth of microorganisms. Bacteria readily attach to surfaces associated with water and drainage systems. Debris within a waste trap also provide bacteria with a surface on which they can attach. Once attached and under favourable conditions, these bacteria will multiply, produce slime like substances and form complex surface associated biofilms.

Bacteria present within a biofilm are more resistant to the action of disinfectants primarily due to them being shielded and protected by other organisms and/or other components of the biofilm. This allows them to proliferate in an environment ideally suited to the acquisition and spread of antibiotic resistance genes. The high cell density and close contact among cells within the biofilm matrix can facilitate horizontal gene transfer and several studies have demonstrated an increased conjugation efficiency in biofilms when compared to free-living bacterial cells.

Hospital sinks and drains can harbour large, multi-species communities and have been identified as environmental reservoirs in outbreaks of *Pseudomonas aeruginosa* and other waterborne pathogens. Increasing evidence from several NHS Trusts suggests they can also harbour multidrug resistant Gram-negative organisms including carbapenemase-producing Enterobacterales (CPE) and, in the absence of alternative scenarios, hospital sinks are increasingly implicated as the source of transmission.

What is less clear is how these bacteria are reaching patients and causing infection. Sink design may play an important role. Tap water flowing directly into the drain hole can disrupt biofilm and disperse droplets containing bacteria up to 1 m from the sink. Ensuring good efficient drainage from a sink, regardless of its design, is also essential. If drainage is slow (e.g. due to blockages), pathogens present in the waste trap, pipework and/or drain can flow back through the system and contaminate sink surfaces thereby facilitating splash dispersal.

Poor penetration and/or the inactivation of disinfectants within the biofilm matrix means well-established biofilms are highly resistant to disinfection. Whilst a variety of treatments have claimed to reduce biofilm in drainage systems, when applied within the clinical setting, results have been variable. CPE can persist in drains despite disinfection with chlorine. Whilst acetic acid has been used to eliminate metallo-β-lactamase-producing *P. aeruginosa* from sink drains, deep seated contamination resulted in further clinical infections, highlighting how putative biofilm can persist.

The eradication of biofilms from hospital drains is difficult and there is a lack of guidance on how to proceed should CPE (or other resistant organisms) be recovered from sinks and/or wastewater plumbing.

## Aims of Research

To utilise a unique laboratory model that incorporates sinks, waste traps and associated plumbing known to be colonised with Enterobacterales (including antibiotic resistant strains) and *Pseudomonas* spp. (including *P.* aeruginosa) and:

1. assess the effectiveness of different chemical disinfectants and modes of delivery.
2. establish where within the sink drainage system disinfectants should be applied (should target) to be most effective in preventing dispersal of organisms
3. determine frequency of disinfection

## Material and Methods

### Model sink system

The Biosafety, Air and Water Microbiology Group (Public Health England Porton) has designed a unique laboratory model (Figure 1) that incorporates stainless steel (base-draining) sinks, ceramic (rear-draining) handwash basins and waste traps, the latter removed from hospital wards. This (previously described) controlled setting has been used to study bacterial colonisation, survival and migration within sink drainage systems and the potential for CPE to detach from drain biofilms and contaminate surrounding surfaces [1].

The system is controlled automatically and, for the purposes of the current study, was programmed to operate each tap for 45 seconds, four-times a day (flow rates ranged from 4.2 L/min to 5 L/min). The water delivered from each outlet passes through a thermostatic mixer valve which blends the hot and cold water so that the temperature of the dispensed water is ~ 41ºC. A waste pump operating at the same time as each tap drains the water into a collecting vessel. Delaying operation of the pump (in this study by 20 seconds) simulates slow (or poor) drainage and allows water to accumulate within the base of the sink.

Figure 1: Experimental model system to study the role of the hospital sink in transmission of infection. Taps and drainage are automatically controlled via a building management system.

## Water quality

Chemical Analysis: Water delivered from the taps was analysed for hardness and free chlorine concentration using a Palin test photometer and was determined to be 241 mg per litre (as $CaCO_3$) and 0.05 mg per litre respectively (n = 4).

Microbiological analysis: Water samples (100 ml) were passed through a 0.2 µm membrane filter. Membranes were transferred to MacConkey Number 3 agar (MAC3; EO Laboratories Ltd, Bonnybridge, UK) and incubated at 37°C for 18-24 hours. No bacterial colonies were isolated (i.e. the concentration of Gram-negative organisms including *Pseudomonas aeruginosa* present within the tap water was < 1 cfu/100 ml; n = 2).

## Contamination of the model system

In March 2020, the study team was required to cease all ‘business-as-usual’ research in order to support PHE’s response to COVID-19. The model system was shut down and was not operated until January 2021 when, for the purposes of this study, it was restarted.

A consortium of bacteria, previously isolated from hospital sinks was suspended in tryptone soya broth (TSB; Oxoid Ltd, Basingstoke, UK) and poured into each sink. The culture was held within each individual sink unit (i.e. drain and waste trap) for several days before being flushed from the system. Waste traps were removed from the sinks and the level of contamination (i.e. biofilm) within the trap and on associated pipework was visually assessed. Thereafter, unless otherwise stated, all sinks were dosed with 10 ml sterile TSB at the end of each working day.

## Monitoring bacterial contamination within the sink waste trap

The type and concentration of Gram-negative bacteria present within the waste trap water was monitored weekly (prior to disinfection) and daily thereafter. Regular monitoring allowed natural variation within the system to be assessed and the effect of disinfection to be determined.

A sterile Stripette or length of sterile tubing was inserted into the drain hole of the base-draining and rear-draining sink respectively. A sterile syringe was attached to the Stripette or tubing and used to withdraw a sample of water from the waste trap. Each sample was serially diluted (10-

fold) in sterile water (prior to disinfection) or validated neutralising solution (post-disinfection) and an aliquot (100 µl) of each dilution ($10^0$ to $10^{-5}$) cultured on MAC3. All plates were incubated at 37°C for 18-24 h.

After incubation, presumptive Gram-negative organisms were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonik MALDI biotyper; Bruker, Bremen, Germany) using the direct transfer method. These isolates also underwent antibiotic resistance profiling using the disc diffusion method (amikacin, ceftazidime, ciprofloxacin, ertapenem, gentamicin and meropenem) following EUCAST guidelines. Once confirmed, Gram-negative colony counts were converted to CFU/ml of waste trap water.

### Dispersal of contaminants during sink usage

To determine if, and when, the dispersal of contaminants is reduced (or otherwise impacted) following disinfection of a sink unit, 10 and 16 MAC3 agar plates were positioned around the perimeter of the base-draining and rear-draining sinks respectively. Bacteria contained within splashes or droplets generated during operation of the tap were deposited on the plates and cultured and identified as described above. The number of Gram-negative bacteria (expressed as splash-forming units (SFU)) was assessed prior to disinfection, immediately after disinfection and daily thereafter.

To confirm that these splashes or droplets contained bacteria originating from the sink unit and not from the tap water, a shallow sterile container was placed at the base of the sink and the tap operated. Splashes were generated as the tap water hit the container and were deposited on the agar plates surrounding the basin. None contained culturable Gram-negative bacteria.

The rate at which water drains from a sink has been shown to impact bacterial dispersal; fast, efficient drainage can help minimise dispersal particularly from rear-draining sinks [1]. In this study, to simulate a 'worse-case' scenario, drainage from all sinks was impaired. This was done by delaying operation of the waste pump by 20 seconds.

Colonisation of the drain strainer or rear-drain hole can result in biofilm formation and a persistent source of contamination. To assess the impact of disinfection on surface-associated contaminants, cotton swabs, pre-moistened with sterile water or validated neutralising solution were used to sample the outer surface of the drain strainer (base-draining sink) and inner surface of the drain hole (rear-draining basin) before and after disinfection.

## Chemical disinfectants

Four products were selected on the basis of active ingredient, formulation and commercial availability. The efficacy of the following was assessed: a chlorine-based (domestic) foaming product (< 5% active chlorine; Mr Muscle drain foamer, SC Johnson, Wisconsin, USA), a commonly used chlorine-based solution (10,000ppm; 1% active chlorine, Haz-Tabs, Guest Medical, Kent, UK), a peracetic acid (PAA)-based foaming disinfectant marketed towards healthcare (0.4% PAA; Clinell Drain Disinfectant, GAMA Healthcare, Hertfordshire, UK) and a PAA (liquid) formulation (Puristeril 340, Fresenius Medical Care, Bad Homburg, Germany). As far as was practicable the concentration of each liquid disinfectant was diluted to that of the active ingredient incorporated within the respective foaming product (as stated in the manufacturers' MSDS).

## Neutralisation of chemical disinfectants

To ensure the efficacy of the disinfectants was not over-estimated, it was essential to neutralise the active ingredient prior to culturing waste trap water or swab samples. If neutralisation is not carried out or is ineffective, residual disinfectant may continue to have an effect during the culturing process (i.e. over an unrealistic contact time) and prevent the growth of the target organism(s). Effective neutralising solutions differ with active ingredient and so knowledge regarding the constituents of a disinfecting product is essential. If this knowledge is lacking (e.g. due to undisclosed 'proprietary' product information) then developing and/or validating appropriate neutralising solutions can be difficult and time consuming.

Five different neutralising solutions (Table 1) were assessed as previously described [2]. Any neutraliser achieving an efficacy and/or toxicity ratio < 0.75 was considered inappropriate for use.

Table 1: Solutions assessed for their potential to neutralise the four chemical disinfectants.

| Solution | Composition (in water) | g/l |
|---|---|---|
| **1** | Dey-Engley neutralizing broth with Tween (Neogen, Lansing, USA) | 78.0* |
| **2** | lecithin | 3.0 |
| | sodium thiosulphate | 3.0 |
| | Tween 80 | 30.0 |
| | catalase | 0.025 |
| **3** | lecithin | 3.0 |
| | sodium thiosulphate pentahydrate | 7.8 |
| | Tween 80 | 30.0 |
| | L-histidine | 1.0 |
| **4** | sodium thiosulphate pentahydrate | 15.69 |
| | Tween 80 | 120.0 |
| | 40% sodium bisulphate | 25.0 |
| | catalase | 0.025 |
| **5** | sodium thiosulphate pentahydrate | 15.69 |
| | Tween 80 | 120.0 |
| | 40% sodium bisulphate | 25.0 |
| | catalase | 0.025 |
| | sodium thioglycate | 10.0 |
| | L-cysteine | 3.0 |

* NB: used at 2-times the recommended concentration

Three of the four disinfectants, when tested at their in-use concentration, could not be effectively neutralised by any of the five solutions. There was insufficient time during the current study to formulate any additional neutralising solutions. Instead, the ability of the five solutions to neutralise any residual disinfectant (i.e. likely to be present post-treatment) was assessed.

Four of the sinks incorporated within the model system were not treated as part of the current study. The chlorine-based foaming product was applied to one of these non-study sinks and allowed to remain *in situ* for the appropriate contact time before being flushed from the system.

Prior to disinfection, the concentration of free chlorine within the waste trap was 0.04 mg per litre. After treatment (i.e. post-disinfection and post-flush), the free chlorine concentration was 3.9 mg per litre indicating that residual disinfectant had been retained within the waste trap at a concentration that could impact subsequent culture results. However, when present at this concentration, the disinfectant could be effectively neutralised.

This analysis was repeated for each of the other disinfectants and all could be effectively neutralised. Consequently, one of the five solutions (solution 2; Table 1) was selected for use in all experiments.

## Disinfection of sinks

Eight different sinks were disinfected as part of this study with each disinfectant being used to treat one base-draining and one rear-draining sink. Liquid disinfectants (500 ml) were poured directly into the sink drain. The foaming disinfectants were applied as per manufacturers' instructions. In all cases, the product was allowed a 15-minute contact time before the tap was operated (45 sec) and the disinfectant flushed from the waste trap and associated pipework.

## Results

### Disinfection of base-draining sinks

Prior to disinfection, the mean concentration of Gram-negative bacteria recovered from samples of waste trap water taken from the base-draining sinks was $9.05 \times 10^6$ cfu/ml (n = 4). Predominant organisms included *Citrobacter freundii* and *Pseudomonas aeruginosa*.

Immediately following treatment with either of the chlorine-based products, colony counts were reduced to below detectable levels (i.e. disinfection achieved a > 4 $\log_{10}$-reduction in culturable Gram-negative bacteria; Figure 2). There was no immediate increase in the number of Gram-negative organisms recovered from the waste traps. However, recovery did increase over time and, following treatment with the chlorine-based foaming product and the chlorine-based liquid, the concentration of Gram-negative bacteria recovered from the waste trap water had increased to baseline levels within 48- and 72-hours respectively (Figure 2).

Immediately following treatment with the peracetic acid (PAA)-based liquid formulation, the number of culturable Gram-negative bacteria recovered from the waste trap water was reduced to below detectable levels but, as with the chlorine-based foam, 48-hours after treatment, bacterial numbers had returned to baseline (Figure 3). In comparison, the PAA-based foaming product had less of an immediate effect but did appear to exert short-term residual activity. Whilst initial treatment achieved an approximate 3.5 $\log_{10}$ reduction in culturable Gram-negative bacteria, a further 0.5 $\log_{10}$ reduction occurred during the 4 hours following disinfection (Figure 3). This effect was not long-lasting and colony counts had returned to baseline within 48-hours.

Seven days after disinfection, the mean concentration of Gram-negative bacteria recovered from samples of waste trap water taken from the base-draining sinks was $6.5 \times 10^6$ cfu/ml (n = 4). Predominant organisms included *Citrobacter freundii*, *Enterobacter* spp and *Pseudomonas aeruginosa*.

Figure 2: The number of Gram-negative bacteria (CFU/ml) recovered from the waste trap water of a base-draining sink following treatment with either a chlorine-based liquid formulation (blue line) or chlorine-based foaming disinfectant (red line). The number of bacteria dispersed from the sinks (splash forming units) during operation of the tap is also presented (circles).

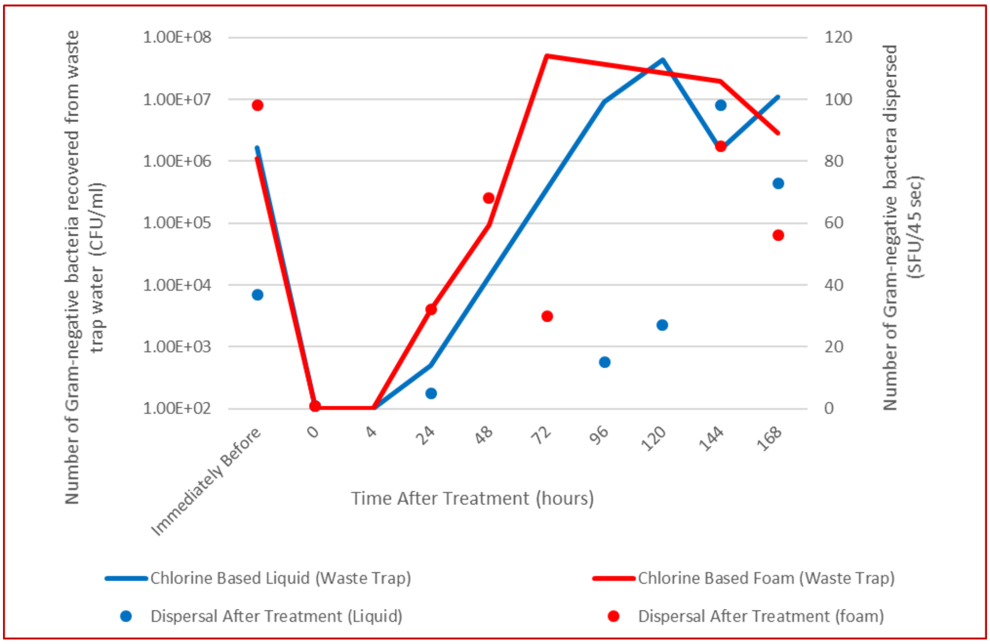
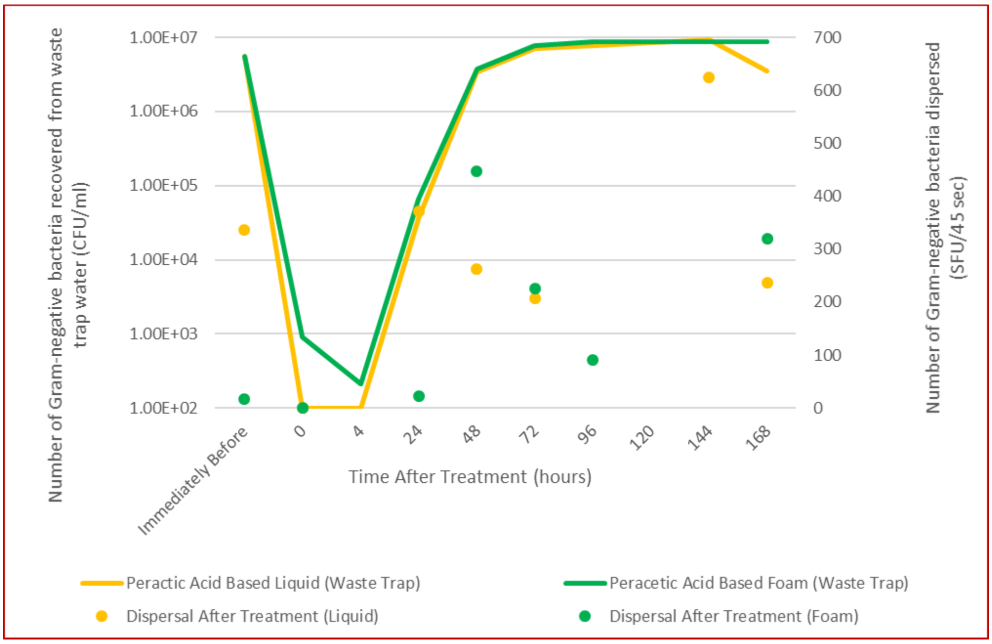



Figure 3: The number of Gram-negative bacteria (CFU/ml) recovered from the waste trap water of a base-draining sink following treatment with either a peracetic acid (PAA)-based liquid formulation (yellow line) or PAA-based foaming disinfectant (green line). The number of bacteria dispersed from the sinks (splash forming units) during operation of the tap is also presented (circles).



## Disinfection of rear-draining handwash basins

Prior to disinfection, the mean concentration of Gram-negative bacteria recovered from samples of waste trap water taken from the rear-draining basins was 1.9 x $10^6$ cfu/ml (n = 4). Predominant organisms included *Citrobacter freundii*, *Klebsiella* spp and *Pseudomonas aeruginosa*.

Immediately following treatment with the chlorine-based liquid disinfectant, the number of culturable Gram-negative bacteria recovered from the waste trap water was reduced to below detectable levels. Whilst there was no immediate increase in colony counts, recovery did increase over time and 48-hours after treatment, bacterial numbers had returned to baseline (Figure 4). Following treatment with the chlorine-based foaming product, the number of Gram-negative organisms within the waste trap was reduced but not eliminated. In the absence of any residual effect, the concentration of Gram-negative bacteria recovered from the waste trap water increased over the 4 hours following disinfection and had returned to baseline levels within 24-hours (Figure 4).

In comparison to the chlorine-based liquid, the chlorine-based foam appeared to be more effective in reducing contamination within the drain. Immediately following treatment, the number of Gram-negative bacteria recovered from the inner surface of the drain hole was reduced by 3.6 and 5.3 $\log_{10}$ values respectively. However, in both cases, re-colonisation of the drain was rapid and bacterial levels had returned to baseline within 24-hours (Figure 4).

Immediately following treatment with the peracetic acid (PAA)-based liquid formulation, the number of culturable Gram-negative bacteria recovered from the waste trap water was reduced to below detectable levels. Colony counts prior to disinfection were unexpectedly low (2.10 x $10^4$ cfu/ml) and so it can only be stated that disinfection achieved > 2-$\log_{10}$ reduction. Recovery increased over time but, in comparison to the chlorine-based liquid (Figure 4), at a seemingly slower rate. The concentration of Gram-negative bacteria recovered from the waste trap water increased to the initial (low) baseline level within 24-hours and the expected level of contamination ($10^6$ cfu/ml) after 72-hours (Figure 5).

When used to disinfect a rear-draining handwash basin, the PAA-based foaming product was the least effective of the products/formulations assessed. Immediately following treatment, the number of culturable Gram-negative bacteria recovered from the waste trap water was reduced by just 2-$log_{10}$ values with colony counts returning to baseline within 4-hours (Figure 5). This product was equally as ineffective in reducing contamination within the drain. Immediately following treatment, the number of Gram-negative bacteria recovered from the inner surface of the drain hole was reduced by 1.5 $log_{10}$ values. In comparison, the PAA-based liquid formulation achieved a > 5 $log_{10}$ reduction but, as with the chlorine-based liquid, re-colonisation of the drain was rapid and bacterial levels had returned to baseline within 24-hours (Figure 5).

Seven days after disinfection, the mean concentration of Gram-negative bacteria recovered from samples of waste trap water taken from the rear-draining sinks was 4.2 x $10^6$ cfu/ml (n = 4). Predominant organisms included *Citrobacter freundii* and *Pseudomonas aeruginosa*.

Figure 4: The number of Gram-negative bacteria (CFU/ml) recovered from the waste trap water of a rear-draining handwash basin following treatment with either a chlorine-based liquid formulation (blue line) or chlorine-based foaming disinfectant (red line). The number of bacteria recovered from the drain (dashed lines) and dispersed from the sinks (splash forming units) during operation of the tap is also presented (circles).

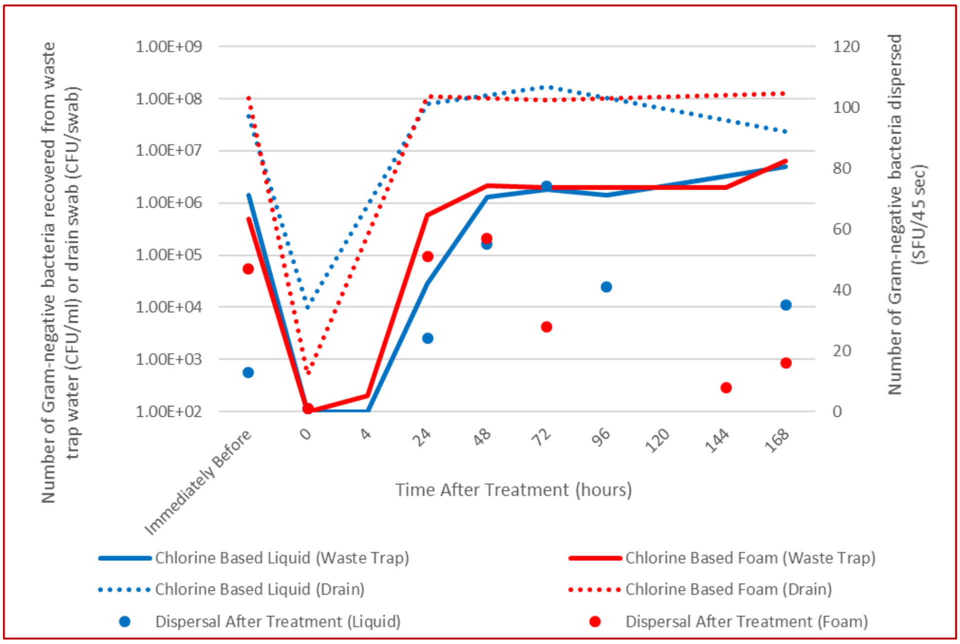
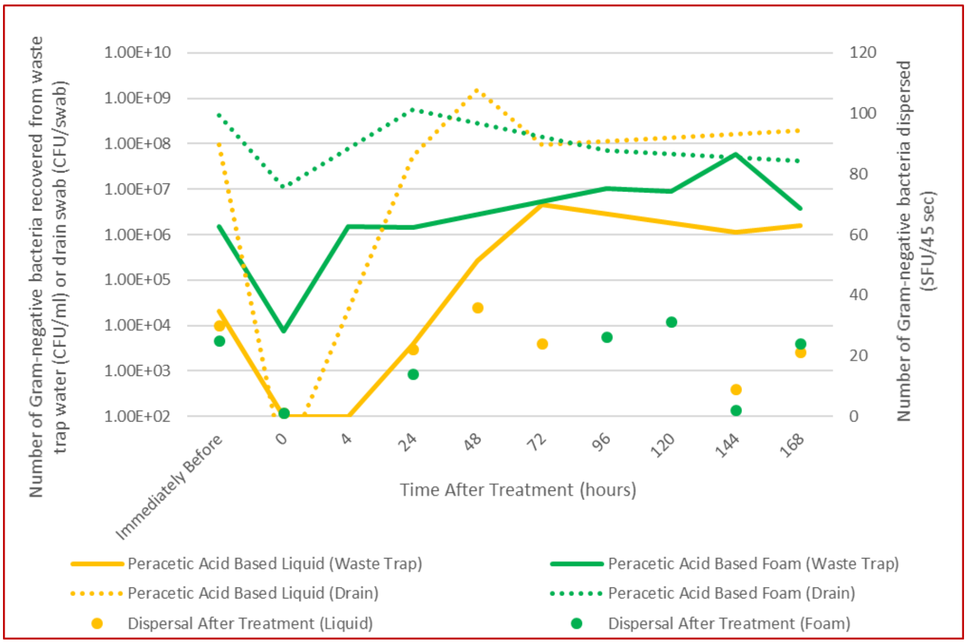


Figure 5: The number of Gram-negative bacteria (CFU/ml) recovered from the waste trap water of a rear-draining handwash basin following treatment with either a peracetic acid (PAA)-based liquid formulation (yellow line) or PAA-based foaming disinfectant (green line). The number of bacteria recovered from the drain (dashed lines) and dispersed from the sinks (splash forming units) during operation of the tap is also presented (circles).




## Reducing dispersal of contaminants during sink usage

Previous studies have demonstrated that dispersal of contaminants can occur if/when water dispensed from the tap directly hits the sink drain and/or strainer. Dispersal from rear-draining sinks is reduced but can occur particularly if drainage is slow.

Prior to disinfection, Gram-negative organisms were dispersed from the base-draining sinks as water dispensed from the tap hit the sink drain and/or strainer. Dispersal varied with sink and, in general, a higher number of 'splash-forming units' were recovered from surfaces surrounding the sinks treated with PAA (Figure 2 and 3). The waste traps (and associated pipework) associated with these sinks were colonised with comparatively heavier (more established) biofilm (Appendix A; Figure D) and this may have accounted for the higher dispersal. Immediately following treatment of the base-draining sinks, and regardless of product, no bacterial dispersal was observed. However, over time and as the concentration of Gram-negative organisms increased within the waste trap, the number of contaminants dispersed to surrounding surfaces also increased to baseline (Figure 3).

Prior to disinfection, and when drainage was impaired, Gram-negative organisms were dispersed from the rear-draining basins. Immediately following treatment with the chlorine- or PAA-based liquid disinfectant, no bacterial dispersal was observed. However, over time the number of contaminants dispersed to surrounding surfaces increased to (or exceeded) baseline. Dispersal varied slightly between sinks and appeared to be influenced by contamination/colonisation of the drain hole with greatest dispersal coinciding with peak contamination levels (i.e. 72-hours (Figure 4) and 48-hours (Figure 5)).

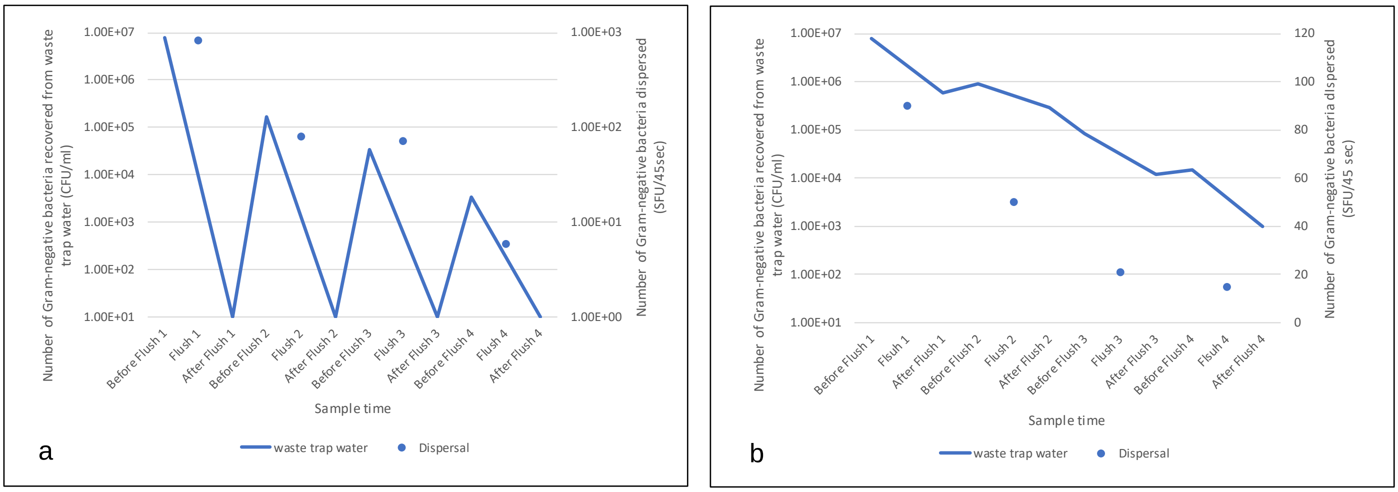
Treating the rear-draining basin with the chlorine-based foam immediately reduced the number of culturable Gram-negative bacteria recovered from the drain hole. Bacterial dispersal was prevented but returned to baseline with rapid re-colonisation of the drain. However, despite contamination levels recovered from the drain hole stabilising over time, the number of Gram-negative bacteria dispersed to surrounding surfaces was observed to decrease (Figure 4). In contrast, the PAA-based foam had little effect upon the number of Gram-negative bacteria recovered from the drain hole or the number of bacteria dispersed (Figure 5).

## Impact of sink use and misuse

Ensuring a sink (or handwash basin) is frequently used can itself reduce the number of contaminants in the waste trap water and can also prolong the effects of disinfection.

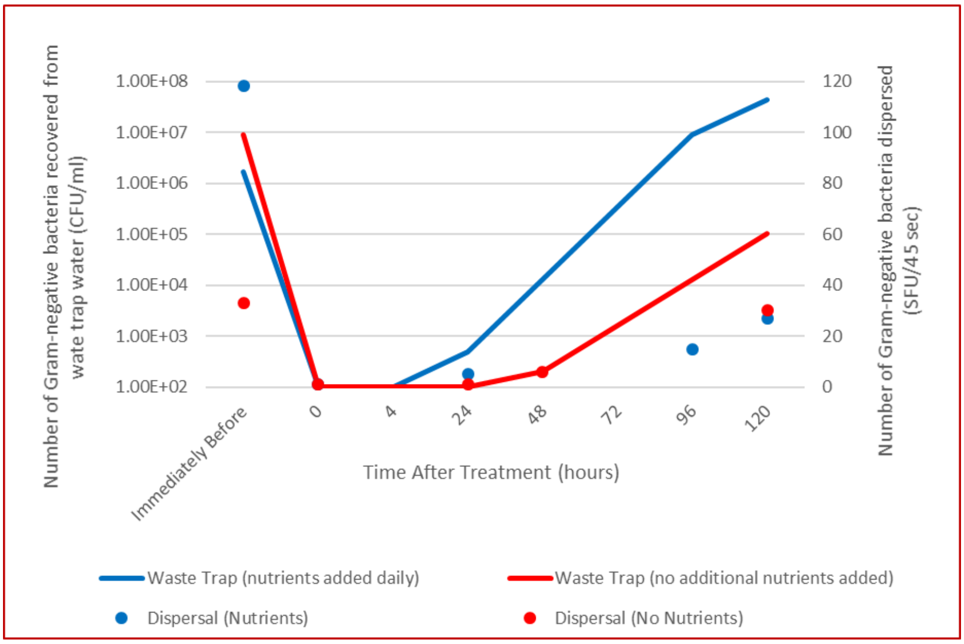
In the absence of disinfection, each time the base-draining sink was ‘used’ (i.e. each time the tap was operated for 45-sec), the number of Gram-negative bacteria recovered from the waste trap water was reduced to below detectable levels. During each subsequent 2-hour stagnation period, bacterial numbers increased to a level approximately 10-100-times lower than that detected before the previous flush (Figure 6a). Whilst ‘use’ of the rear-draining basin did not remove all culturable Gram-negative bacteria from the waste trap water, each time the tap was operated, the concentration of Gram-negative bacteria reduced 10-fold (i.e. by ~1-log value; Figure 6b). In both cases, as the number of bacteria declined, bacterial dispersal also decreased.

Figure 6: Effect of tap (sink) usage on the number of Gram-negative bacteria (CFU/ml) recovered from the waste trap water of a base-draining sink (a) and rear-draining handwash basin (b). The tap was operated once (for 45 sec) every 2 hours. Samples were taken immediately before and immediately after the tap was operated. The number of bacteria dispersed from the sinks (splash forming units) during operation of the tap is also presented (circles).




Inappropriate disposal of liquid waste can introduce nutrients to the waste trap and facilitate bacterial survival and growth. In this study, nutrients were added to the sinks once each day to simulate inappropriate usage. Experiments were also carried out in the absence of additional nutrients. Immediately following treatment with the chlorine-based liquid formulation, the concentration of Gram-negative bacteria within the waste trap of a base-draining sink was reduced to below detectable levels. Colony counts returned to baseline with 3 days. If nutrients were not introduced, the impact of disinfection was longer-lasting (Figure 7). Despite this however, periods of stagnation caused by lack of use (e.g. between 48- and 96-hours post-treatment; Figure 7) led to rapid re-colonisation.

Figure 7: The number of Gram-negative bacteria (CFU/ml) recovered from the waste trap water of a base-draining sink following treatment with a chlorine-based liquid formulation. Following treatment, the waste traps were either exposed to regular (daily) dosing of nutrients (blue line) or were not (red line). The number of bacteria dispersed from the sinks (splash forming units) during operation of the tap is also presented (circles).



## Conclusions

### Efficacy of chemical disinfection and modes of delivery

- Results suggest that use of a chlorine-based (10,000ppm (1%)) or peracetic acid (PAA)-based (0.4%) liquid disinfectant can reduce the concentration of planktonic bacteria within a sink waste trap. In this study, the waste traps (and associated plumbing) were colonised with moderate biofilm and treatment achieved at least a 4-log reduction in culturable Gram-negative bacteria. However, despite reducing visible contamination (Appendix A; Figure A and C) neither liquid disinfectant eliminated the colonising biofilm and bacterial levels returned to baseline 48 - 72 hours later.
- It has been suggested that foam-based products may be more effective in reducing bacterial contamination within sinks and drains than liquid disinfectants. It was hypothesised that a foaming product would allow greater surface coverage and a longer contact time between the surface and active agent(s). However, in this study the foam-based products were the least effective.
    - The PAA-based foaming product comprises granules that are poured into running tap water. Once they reach the waste trap, they foam-up covering the inner surface of the trap. The manufacturer suggests a contact time of at least 15-minutes, but during the current study, no foam was observed to flow up from the waste trap meaning there was little to no disinfection of the drain. This was particularly evident when the product was used to treat the rear-draining sink (Figure 5).

    - In contrast, the chlorine-based foaming product did foam-up and flow back into the basin and, unlike the chlorine-based liquid, was retained within the rear-drainage outlet (drain hole) until flushed from the system. This comparatively longer contact time likely accounted for its increased efficacy in reducing Gram-negative bacteria within the drain (Figure 4). Nonetheless, there was little residual action and re-colonisation of the drain and waste trap was rapid.

### Reducing the dispersal of contaminants during sink usage

- Results suggest the dispersal of contaminating organisms can be reduced (and perhaps prevented) immediately following disinfection (regardless of product). However, as colonisation within the waste trap (strainer or drain hole) increases over time, dispersal to surrounding surfaces will likely increase.

- Prior to disinfection, the mean number of Gram-negative bacteria recovered from waste trap water taken from the different sinks was 5.5 x $10^6$ CFU/ml (n = 8). Previous work has demonstrated that when contaminants are confined to the waste trap dispersal is minimal, regardless of drain position [1]. However, formation and presence of biofilm within and/or beyond the waste trap is associated with increased risk of dispersal.
    - In the current study, the highest numbers of bacteria were dispersed from base-draining sinks with strainers contaminated with Gram-negative bacteria (>$10^4$ CFU) and/or associated with the heaviest biofilm (Appendix A; Figure D). The PAA-based disinfectants had little effect on bacterial dispersal (Figure 3) and further work is investigating the role and disinfection of the strainer and the efficacy of alternative formulations against established biofilm.

    - Dispersal from rear-draining sinks is reduced but can occur, particularly if drainage is slow. In this study, drainage was impaired and Gram-negative bacteria were dispersed from all rear-draining sinks. Highest dispersal appeared to correlate with contamination levels within the rear-drainage outlet. None of the chemical disinfectants assessed effectively disinfected this area of the sink. Alternative formulations are currently being investigated.

## Frequency of disinfection

- Sinks, drains and waste traps can become heavily contaminated and, in this study, disinfection had little effect in reducing heavy (established) biofilm (Appendix A; Figure D).
- Ensuring a sink (or handwash basin) is frequently and appropriately used (i.e. is not used to discard liquid waste) may help prevent biofilm formation, enhance chemical disinfection and allow for longer-term effect. However, results from this study would suggest that chemical disinfectants (if effective) are a short-term solution and to achieve a sustained reduction in bacterial numbers (and as a result dispersal), would likely require frequent (e.g. daily) application.
- Sink design continues to evolve, and recent models have been shown to continually reduce/prevent bacterial dispersal [3]. For longer-term solutions (ward refurbishments, new builds) installation of these innovative designs should be considered.

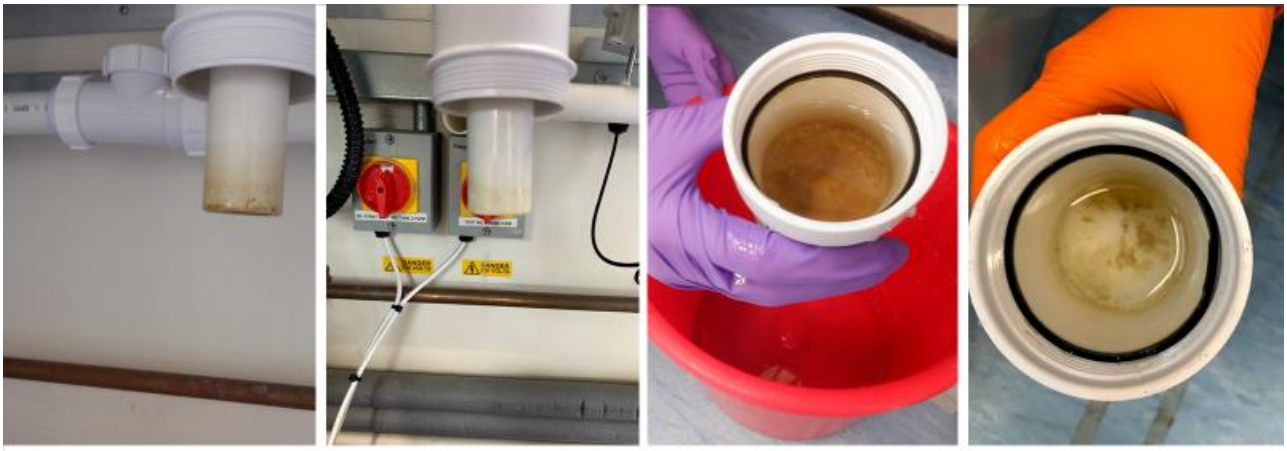
## References

1. Aranega-Bou, P, George RP, Verlander NQ, Paton S, Bennett, A and Moore G. Carbapenem-resistant Enterobacteriaceae dispersal from sinks is linked to drain position and drainage rates in a laboratory model system. *Journal of Hospital Infection*, 2019; 102: 63-69
2. Sutton SVW, Proud DW, Rachui S and Brannan DK. Validation of microbial recovery from disinfectants. *PDA Journal of Pharmaceutical Science and Technology*, 2002; 56:255-266
3. Aranega-Bou, P, Cornbill C, Verlander NQ and Moore G. A splash-reducing clinical handwash basin reduces droplet-mediated dispersal from a sink contaminated with Gram-negative bacteria in a laboratory model system. *Journal of Hospital Infection*. In Press

## Appendix A

### Visual inspection of waste traps before and after 7-day disinfection and monitoring period

A.
Base-draining sink: before and after treatment with a chlorine-based liquid disinfectant



B.
Rear-draining sink: before and after treatment with a chlorine-based foaming disinfectant

C.
Rear-draining sink: before and after treatment with a peracetic acid-based liquid formulation

D.
Base-draining sink: before and after treatment with a peracetic acid-based foaming disinfectant