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Sodium dichloroisocyanurate: a promising candidate for the disinfection of resilient drain biofilm

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S U M M A R Y

Background: Biofilms are complex multicellular communities of microorganisms embedded within a protective matrix which confers resistance to various antimicrobials, including biocides. Biofilms can cause a range of human diseases and are responsible for 1.7 million hospital-acquired infections in the US annually, providing an economic burden of $11.5 billion in treatment costs. Biofilm contained within drain and plumbing systems may contain pathogenic viruses and bacteria which pose a significant risk to patient safety within healthcare environments.

Aim: The aim of this study was to determine if three hospital-grade disinfectants (sodium dichloroisocyanurate, peracetic acid and sodium hypochlorite) were capable of killing microorganisms within biofilm, and thus, determining their potential as candidates for drain biofilm disinfection.

Methods: *Pseudomonas aeruginosa* biofilms were cultivated using the CDC biofilm reactor, a standardised method for determining disinfectant efficacy against biofilm within the United States of America. Each disinfectant was tested using a one-minute contact time, using the highest concentration available on the product label.

Findings: The sodium dichloroisocyanurate product was successful in killing biofilm microorganisms, resulting in a log reduction of ≥ 8.70. Peracetic acid reduced biofilm by 3.82 $\log_{10}$ units, followed by sodium hypochlorite, which produced a reduction of 3.78 $\log_{10}$ units.

Conclusions: The use of a highly effective disinfectant with proven biofilm efficacy can help ensure patient safety and reduce infection levels. Drains and plumbing systems provide a reservoir for potential pathogens and biofilm; thus, drain disinfection is critical in reducing the instance of hospital-acquired infections. Sodium dichloroisocyanurate may provide a reliable solution for drain applications and subsequently, patient wellbeing and safety.

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Abbreviations: °C, Degree Celsius; CDC, Centres for Disease Control; CFU, Colony forming units; D/E broth, Dey-Engley neutralising broth; hr, Hour; HWW, Hospital wastewater; ICU, Intensive Care Unit; IFU(s), Instruction(s) for use; ml, millilitres; rpm, Revolutions per minute; s, second; TSA, Tryptic soy agar; TSB, Tryptic soy broth.

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Introduction

Biofilms are prehistoric modes of growth and survival which may be employed by over 99% of all microorganisms [1] and date back 3.5 billion years [2]. It is estimated up to 80% of all bacterial cells on earth currently reside within biofilm [3]. Biofilm formation is a phenotypic adaptation that occurs in response to certain environmental stressors, such as low nutrient density or high shear conditions [1,4] and thus does not require any genetic alteration.

Biofilms can be defined as a robust population of microbial cells contained and embedded within a self-produced matrix layer primarily composed of extra polymeric substances (EPS) [4]. The EPS layer typically includes polysaccharides, eDNA, proteins, amyloids, pili, fimbriae, and a variety of other cellular components [1,5], which bind to a living or non-living surface. The EPS layer, along with size, antibiotic tolerance, persister cells and increased mutation rates, provides a layered defence to the pathogens within [6] making the biofilm virtually impenetrable to antimicrobials. Biofilm can form almost anywhere [2] and are responsible for the slimy texture often found on areas such as teeth, pool surfaces and within sinks and drains.

Despite its foremost function being that of survival, biofilm has been aptly described as a *primary virulence factor* [7]. This is because the physical properties it confers, while transient and impermanent [6], give rise to populations of antibiotic-resistant bacteria [8]. Bacteria that live within a biofilm have been shown to exhibit increased antibiotic resistance, up to 1000-fold, when compared to the equivalent planktonic cells [9]. Furthermore, biofilms are often multi-species, resulting in a wider availability of genetic elements for potential sharing between the bacteria within, increasing and accelerating the chance of resistance acquisition [10].

Alongside alterations to antibiotic susceptibility, biofilm may also render disinfectants ineffective, particularly if the biofilm initially formed as a result of stress caused by the presence of disinfectant within the local environment. Similar to antibiotics, many disinfectants are incapable of penetrating and diffusing across the thick EPS layer. Disinfectants that succeed in crossing this barrier may not possess the ability to kill the biofilm due to the physiologically altered state of the cells within. Disinfectants which are incapable of complete inactivation of viable cells may assist in the potential acquisition of biocidal resistance. Biocidal resistance may result in planktonic cells which are immune to disinfectants and thus, regular infection prevention protocols, however evidence highlighting acquired resistance of bacteria to high-level disinfectants is lacking. There is also evidence to suggest that exposure to biocides in such a manner may assist in the acquisition of antibiotic resistance genes by the bacteria [11]. Furthermore, several bacterial species are capable of surviving antimicrobial treatment due to the presence of persister cells. Persister cells, while only comprising approximately 1% of the overall population of cells within a biofilm, are arguably the most dangerous. These cells grow at a significantly slower rate than their counterparts and are metabolically inactive. Persister cells remain in a state of dormancy, halting biosynthesis within the cell and conferring an extreme tolerance to antibiotics, as the metabolic processes which are targeted by antibiotics is no longer active. Therefore, while it may appear a biofilm has been eradicated successfully, if these cells remain, they may recommence metabolic activity once favourable conditions become available, initiating the reestablishment of the biofilm [12].

Biofilm pose a serious infection risk because, while ultimately attached to a surface, biofilm is not stable in nature and cells can break off and become planktonic, which may lead to infection [1,13,14]. Since these cells are equipped with a stronger arsenal of defence, resulting infections may be severe or possibly fatal to already ill and immunocompromised hosts. Ultimately, the cells that originally established the biofilm may have been largely harmless and non-pathogenic, but those who exit generations later may be completely untreatable [4,15]. As a result, there has been a global rise in nosocomial infections such as urinary tract infections, respiratory pneumonia, and wound infections [16,17]. Bacteria which would otherwise be non-pathogenic, combined with an abundance of susceptible hosts, can lead to dangerous infections [18]. A broad-spectrum disinfectant capable of penetrating multiple layers of the biofilm structure, including a variety of pathogenic species and the population of persister cells within, would be favourable to ensure total eradication of the biofilm preventing further resistance acquisition and infection spread.

Increases in biofilm resistance impacts economic, health and medical burdens. Bacteria are acquiring resistance faster than antibiotics can be discovered and developed with multidrug-resistant (MDR) pathogens costing the United States an estimated 4.6 billion USD annually [19]. Biofilm growth and subsequent antibiotic resistance acquisition may be inhibited by eradicating the problem at the source. Implementation of infection prevention practices that utilise broad-spectrum disinfectants capable of killing the variety of microbial species, including MDR species that may reside in biofilm, is critical.

However, while the eradication of biofilm and implementation of stringent disinfection protocols is simple in theory, one source of microbial biofilm which is often overlooked is hospital drain biofilm. Hospital drain contamination has garnered much attention in recent years. While it may be relatively simple to kill biofilm present in drain outlets such as plugholes, it is oftentimes present at unreachable depths within the plumbing system and thus can easily spread back towards outlets where patient exposure can occur [20—22]. Splashing in particular can be problematic, as the bacteria-laden water may end up on local surfaces and allow for further transmission, ultimately leading to infection and increased healthcare associated infections (HAIs) [23,24]. Studies carried out in hospital settings show that sink drains maintain the highest burden of antibiotic-resistant bacteria amongst all other surfaces, with *P. aeruginosa* consistently the most prominent species found in Intensive Care Unit (ICU) sink drains [25]. Therefore, an effective disinfectant product that could be poured into a drain system, accessing these hard-to-reach areas, may provide a simple solution for the management and control of drain biofilm.

The aim of this study was to determine if three hospital-grade disinfectants were capable of killing microorganisms within biofilm, and as such, if they could be used as potential drain disinfectants. Biofilm was cultivated using the CDC biofilm reactor and tested against peracetic acid (PAA), sodium hypochlorite (bleach), and sodium dichloroisocyanurate (NaDCC), prepared as per their instructions for use (IFUs). The

CDC bioreactor has been authorised as a standardised method for biofilm growth and treatment testing in the United States of America. Each of the disinfectants were tested using a one-minute contact time. This timeframe is achievable if these disinfectants were to be used for drain disinfection applications, that is, pouring the disinfectant down the affected drain outlet into the plumbing system. If successful, these disinfectants could provide a solution for resilient drain biofilm.

Materials & methods

The method for the cultivation of biofilm using the CDC biofilm reactor is based on the American Standard Test Method ASTM E2562-22 — *Standard test methods for Quantification of Pseudomonas aeruginosa, grown in high shear and continuous flow using the CDC biofilm reactor* and is outlined below.

Test products

Three disinfectant products were evaluated for efficacy (log_{10} reduction) against *P. aeruginosa* biofilms and prepared as per the highest concentration available on their respective IFUs. Neutralisation of all disinfectants with Dey-Engley broth was confirmed by completing a Neutralization Confirmation Test (NCT). At timed intervals, 4mls of disinfectant was added to 36ml of neutralizer and briefly mixed. *Pseudomonas aeruginosa* (diluted to 10^6 CFU/ml) was added and held at room temperature for 15 minutes. After the contact time, the solution was vortexed and 10-fold dilutions were prepared. Spread plates were prepared with 0.1ml aliquots and incubated at 37±2°C for 24 hr. If the recovered number of CFUs from the NCT are within 0.5 log_{10} units of the test culture titre, effective neutralisation is verified.

Preparation of CDC reactor

P. aeruginosa ATCC 15442 overnight cultures were prepared by inoculating 10ml of 300mg/L tryptic soya broth (TSB) with a loop of pre-streaked colonies, alongside a media control which received no inoculation. The required equipment and reagents were prepared, cleaned, and sterilised accordingly in advance of testing.

CDC reactor batch phase

The effluent tube of the reactor was clamped to prevent leakage of the batch medium. 500ml of 300mg/L TSB batch phase growth medium was aseptically added to the reactor by removing one of eight rods from the reactor top and carefully pouring the medium through the opening slot, before reinserting the rod, ensuring the rod alignment pin is secured back into the notch on the reactor top. The reactor was placed onto a magnetic stir plate and the flow break clamped upright using a retort stand. All other tubing was clamped and covered with aluminium foil (Figure 1). The overnight culture was vortexed before 1ml was added into the reactor via an available rigid stainless-steel inoculation port on the reactor top. The stir plate was set to a rotational speed of 125rpm, and the reactor incubated for 24hr at a room temperature of 21±2°C. Additionally, streaking of the inoculation culture was carried out to ensure culture purity and Miles and Misra technique was used to determine the concentration of the culture.

CDC reactor continuous stirred-tank reactor phase

20L of continuous growth medium, contained within a reactor carboy, was prepared at 100mg/L and sterilised. The influent tube was aseptically connected from the carboy to the reactor and the media pumped continuously into the reactor to achieve a 30-minute residence time based on the reactors operating volume. The effluent tubing was aseptically attached to a waste carboy. Pumping was continued for 24 hr at a room temperature of 21±2°C and a rotational speed of 125 rpm which allowed for a continuous flow of media in and out of the reactor.

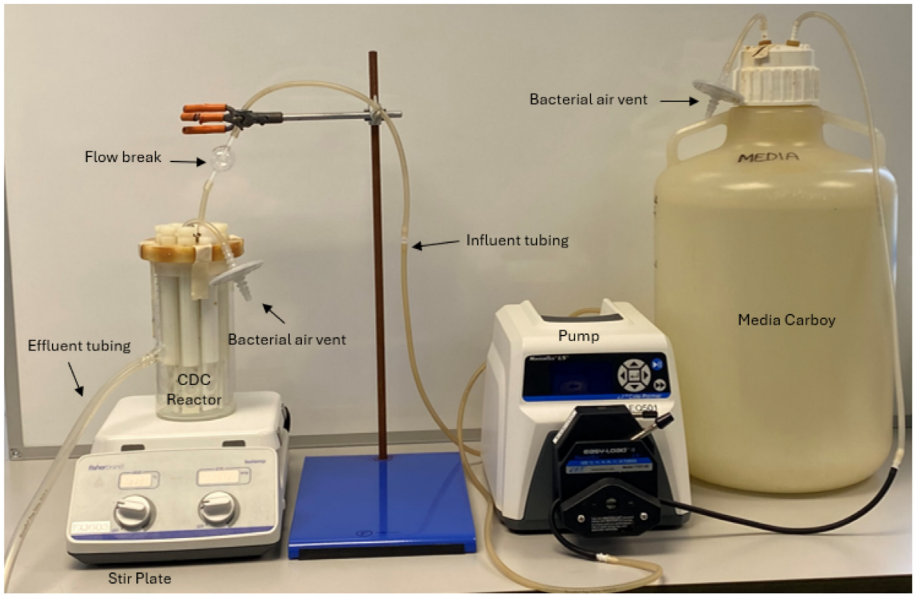

Figure 1. Illustrative CDC Reactor Setup.

Biofilm efficacy testing

Each rod, containing three coupons, was assigned to a different disinfectant. Rods were dipped once into sterile deionised water to remove planktonic cells and gently tapped into a sterile empty Petri dish to remove excess water. The coupons were transferred to individual 50ml falcon tubes containing pre-inserted sterile splashguards using a screwdriver which was flame sterilised and disinfected with isopropyl alcohol. The splashguards were removed, and 4 ml of treatment was added. 4ml of sterile deionised water was used as a control. The tubes were gently tapped to ensure that no air bubbles were present around the coupons such that maximum surface contact could occur. Once the contact time had elapsed (Table I), 36 ml of Dey-Engley (D/E) neutralising broth was added to neutralise the disinfectant and thus halt the disinfection. The lid was screwed back on, and the tube shaken vigorously. Once testing was complete, any remaining biofilm was removed from the coupons via 30s intervals of vortexing and sonication at 50kHz, which was repeated once before one final round of vortexing.

Determining CFU/ml post-treatment

Remaining bacteria were quantified using standard serial dilution and spread plate techniques on tryptic soya agar (TSA) plates. This was performed in triplicate. Plates were incubated at 37±2°C for 24 hr. Results were recorded and CFU/ml for each was calculated. For each disinfectant tested, a total of two individual CDC reactor runs were carried out, resulting in two rods and six coupons per disinfectant tested.

Results

Hospital-grade NaDCC, PAA and bleach products were tested against *P. aeruginosa* biofilm at a contact time of one minute and at concentrations outlined in Table I.

The NaDCC product was successful in killing biofilm microorganisms, resulting in a total of $\geq$8.70 $\log_{10}$ reduction. On average, PAA reduced biofilm by 3.82 $\log_{10}$ units, followed by bleach, which had the lowest reduction at 3.78 $\log_{10}$ units (Table II, Figure 2). As seen in Figure 2, *P. aeruginosa* biofilm grew to 8.70 $\log_{10}$ units.

Discussion

Of the three disinfectant products, NaDCC alone was capable of the greatest log reduction ($\geq$8.70 $\log_{10}$ reduction), while PAA and bleach were much weaker in terms of biofilm kill (3.82 and 3.78 $\log_{10}$ reduction respectively). It is apparent that NaDCC is a highly efficacious disinfectant that is capable of eradicating stubborn *P. aeruginosa* biofilm after only one-minute. NaDCC is commonly used as an effective drinking water disinfectant and is popular due to its wide spectrum efficacy, availability, cost effectiveness, and safety. In water, NaDCC dissolves to release hypochlorous acid as free available chlorine. Hypochlorous acid functions well as a biocide due to the fact that it is structurally similar to water, along with an electrically neutral charge; thus, it readily diffuses across the cell wall in a similar fashion to water, allowing it to kill the bacteria or biofilm from within [26]. Moreover, the equilibrium of available chlorine and remainder bound in mono- or dichloroisocyanurate ensures continuous efficacy with increasing bacterial contamination [27].

P. aeruginosa was chosen as the representative species for testing due to its prominence in biofilm within the drains, sinks and showers of hospitals and other healthcare facilities [28,29]. *P. aeruginosa* is a Gram-negative, aerobic, rod-shaped bacterium [30] that is opportunistic in nature and can cause serious nosocomial infections. Hosts who suffer from cystic fibrosis and other chronic lung conditions are particularly prone to colonisation by *P. aeruginosa* in the lungs [31]. It can form bright green or pale indistinct colonies [32], and its biofilm is extremely thick and slimy. It has also been classified as an

Table I
Summary of disinfectant products tested. *(Table layout adapted from* [22]*)*

Disinfectant	Form	Active ingredient	Concentration of active ingredient (ppm)	Contact time (seconds)
NaDCC	Liquid (tablet dissolved in DI water)	Sodium dichloroisocyanurate	4000	60 ± 10
PAA	Liquid (granules dissolved in DI water)	Peracetic acid	2000	60 ± 10
Bleach	Liquid (concentrated solution diluted in DI water)	Sodium hypochlorite	2400	60 ± 10

Table II
Results from testing the disinfectants against *P. aeruginosa* biofilm

	CFU	Mean LD	LOGR	Average LD	Average LOGR	Standard deviation
NaDCC (1)	0×10^0	0.00	8.92	0.00	$\geq$8.70	0.0000
NaDCC (2)	0×10^0	0.00	8.47			
PAA (1)	7.56×10^4	5.08	4.04	4.98	3.82	0.1414
PAA (2)	1.2×10^5	4.88	3.60			
Bleach (1)	5.02×10^5	5.70	3.22	4.91	3.78	1.1137
Bleach (2)	1.33×10^4	4.12	4.35			
Control (1)	8.32×10^8	8.92	0.00	8.70	8.70	0.3182
Control (2)	2.98×10^8	8.47	0.00			

*LD = log density, LOGR = Log reduction, (1) and (2) refer to individual replicates.

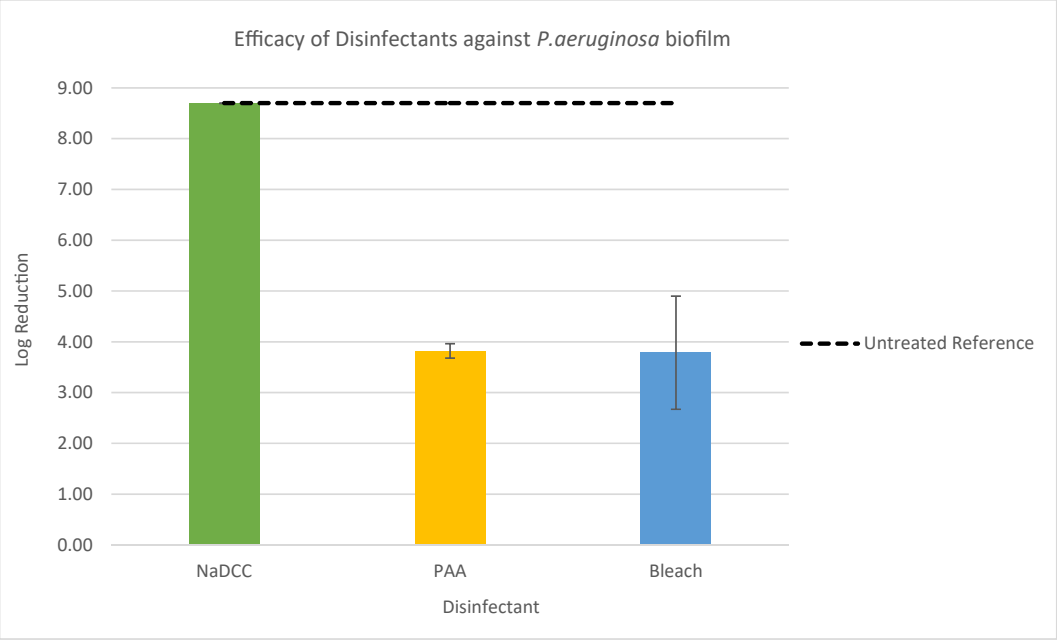

Figure 2. Efficacy of disinfectants against *P. aeruginosa* biofilm cultivated in the CDC reactor.

ESKAPE pathogen, alongside *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneuomoniae, Acinetobacter baumannii* and *Enterobacter* spp., a group of bacteria characterised by increased levels of both virulence and antimicrobial resistance [33,34]. Multi-Drug Resistant (MDR) *P. aeruginosa* has been deemed a serious threat by the CDC, resulting in an estimated 32,600 infections and 2,700 deaths in 2017 alone, accompanied by a substantial 767 million USD in healthcare costs [35]. The presence of *P. aeruginosa* in sink drains may cause infection due to the combination of bacteria-laden aerosols resulting from splashing and the accumulation of bacteria in slow-draining or clogged sinks [36].

Many studies have been carried out to investigate the extent and risk of contamination present in hospitals, and the microbial profile of drains can comprise a cohort of different bacterial species. Hospital wastewater (HWW) has been described as a *cesspool of microbial genome(s)* by Mapipa *et al.*, who studied the presence of *P. aeruginosa* in HWW in Eastern Cape, South Africa. It was found that despite low recovery, multiple virulence factors and occasional antibiotic resistances were found in the recovered samples [37]. Another study found that biofilm present on patient room shower hoses contain a multitude of species, including *Mycobacteria, Erythrobacter, Sphingomonas* and *Novosphingobium*, which are easily transmitted to patients due to their location [38].

The transmission of Gram-negative nosocomial infections to hosts from contaminated toilets has also been demonstrated experimentally [39]. A series of 57 swabs taken from high touched areas in Brazilian hospitals detected ESKAPE pathogens in over half of samples taken and detected biofilm on all swabs, some of which contained MDR microorganisms [40]. Brazilian HWW containing MDR *P. aeruginosa* has been found to be discharged directly into an urban stream used by local populations for bathing and clothes washing [41]. Another study focusing on contamination of an ICU which undergoes regular strict environmental cleaning procedures found 15 different bacterial phyla, including Actinobacteria, Bacteroidetes and Proteobacteria [18]. Additional studies of ICU ward contamination have all reported both bacterial load and biofilms, including MDR species, on multiple surfaces such as bed rails, stethoscopes, ventilators, and handwashing sinks [42—45]. ICU-acquired Gram-negative bacterial infections were shown to be significantly reduced after the removal of sinks from the area [46], underpinning the danger contaminated drains pose to critically ill patients. Gram-negative colonisation is also associated with patients presenting with burns and other types of wounds. A systematic review of antimicrobial efficacy against biofilm determined that many of the current intervention strategies for burn patients may also be applicable to the hospital equipment and surfaces which are interacting with the patient [47]. Interestingly, the authors noted that sinks, drains and toilets are key surface areas which should be included in cleaning and disinfection routines to reduce the risk of patient colonisation [47].

This study determined that three hospital-grade disinfectants, when prepared as per their IFUs (Table I), demonstrated varied efficacy on *P. aeruginosa* biofilm. Each of the three disinfectants were prepared as per the guidance provided on each product label (IFU), ensuring that the highest concentration was used for each disinfectant product. Although some variances were noted between product concentrations, this study aimed to replicate real-life product usage, therefore each of the disinfectant products were prepared as per the label instructions. A one-minute contact time was chosen for all disinfectants used. When used as a drain disinfectant, it is hypothesised that each disinfectant product would be prepared as per the IFU (i.e., the product concentration IFUs), and the resulting solution would be poured down the drain outlet to maintain the desired contact time. A one-minute contact time was chosen as a worst-case scenario, assuming that each product would be poured into a drain system and a short contact time may be achieved.

As per the methods used throughout this study, NaDCC was successful in killing *P. aeruginosa* biofilm with a one-minute contact time. Therefore, NaDCC could be used by slowly pouring the solution into the drain outlet for one minute, helping to reduce and eradicate the presence of *P. aeruginosa* biofilm within the drain system. Successful drain disinfection would decrease the risk of transmission and infection from the drain system, allowing for safer care of patients in healthcare environments.

It is hypothesised that the use of an effective disinfectant would prevent drain biofilm spread, and in-turn reduce the risk associated with splashback onto local surfaces. As stated in literature, the spread of *P. aeruginosa* to the environment via HWW is a major concern, which could be reduced or prevented entirely through treatment with an effective disinfectant. This would significantly reduce the MDR planktonic cells emerging from biofilm-laden drain surfaces. Regular disinfection with NaDCC could prevent the re-establishment of these biofilms. Additionally, lack of biofilm within the plumbing system would result in overall cleaner water. The results from this study would also suggest that NaDCC could have an effect on persister cells found within the biofilm. No growth was observed from the NaDCC-treated biofilms despite plating on nutrient-rich agar and incubation at optimal temperatures, indicating that NaDCC is capable of eradicating all viable cells within the biofilm, including these stubborn persister cells.

This study has potential limitations. The method for determining disinfectant efficacy was based on the American Standard Test Method ASTM E2562-22. Although this method is standardised, real-life drain systems would be subject to a range of organic challenges which is not captured within this study. Similarly, the action of pouring a disinfectant on a biofilm laden coupon, may not reflect the action of pouring a disinfectant into a drainage system. An important point to consider, is the type of disinfectant used for drain applications. The addition of a surfactant to a disinfectant formulation may aid in achieving a longer exposure time, due to the adherence of the foam to the inner surfaces of the drainage system. Further testing in real-life drainage systems would aid in determining the effectiveness of NaDCC for drain disinfection.

Disinfection is of utmost importance within healthcare environments due to the abundance of immunocompromised and/or elderly patients, and oftentimes standard infection prevention and disinfection protocols are insufficient [40,48]. The use of a highly effective disinfectant with proven biofilm efficacy can help to ensure patient safety and reduce infection levels. Biofilm pose a significant threat to patient safety, due to their resilience and perseverance, even when challenged with the toughest cleaning protocols and disinfectant products. Ineffective killing of biofilm can oftentimes facilitate the development of biofilms which exhibit resistance to disinfectants. Resistance and further spread provide the perfect combination for the rise of genetically altered bacterial populations, within which antibiotic resistance will likely be present. Reinfection from these biofilms can thus result in untreatable, possibly fatal infections, and further increase the ongoing issue of antibiotic resistance. Since the screening and subsequent trialling of new antimicrobial compounds can take decades to complete, an alternative, broad-spectrum solution with the ability to combat resistant bacterial populations and help to reduce bacterial biofilm would be a welcome one, with NaDCC a promising candidate. Further studies in real-life hospital conditions could establish the use of NaDCC to combat stubborn drain biofilm.

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Author contributions

Abbie Martin: Methodology, Investigation, Validation, Formal analysis, Writing — Original draft, Writing — Review & Editing. **Natasha Doyle:** Conceptualization, Methodology, Formal analysis, Writing — Original draft, Writing — Review & Editing, Resources. **Tom F. O'Mahony:** Conceptualization, Writing — Review & Editing, Supervision, Project administration, Resources.

Conflict of interest statement

AM, ND and TO'M are employees of Kersia. As employees of Kersia, all authors have a financial relationship with Kersia. Kersia manufactures a wide range of products, including products which have sodium hypochlorite, peracetic acid and sodium dichloroisocyanurate as their active ingredients. As representatives of a disinfectant manufacturer, the authors have chosen to test a wide range of disinfectants to avoid any conflict of interest including some of the most commonly used disinfectants. The authors are aware that there are other disinfectant chemistries which may also provide positive results when tested against bacterial biofilm.

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Ethics

Not required.

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