Journal of Hospital Infection 162 (2025) 121–126

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Short Report

Biofilms and antimicrobial resistance in healthcare: evaluating chlorine dioxide as a candidate to protect patient safety

P. Norville [a,*], **S. Dangleben** [b], **S. Hardy** [b]

[a] *Norvate Limited, Newport, UK*
[b] *Tristel Solutions Limited, Newmarket, UK*

ARTICLE INFO

Article history:
Received 30 January 2025
Accepted 21 April 2025
Available online 22 May 2025

Keywords:
Biofilms
Antimicrobial resistance
Healthcare-associated infection
Disinfection

SUMMARY

Healthcare-associated infections (HAIs) caused by the transmission of multidrug-resistant organisms (MDROs) from contaminated surfaces are a major challenge for healthcare organizations. The presence of biofilm on surfaces makes effective environmental decontamination difficult to achieve and exacerbates antimicrobial resistance (AMR). In this study the performance of various chlorine dioxide-based disinfectants against a panel of MDROs, and biofilms formed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, was evaluated. All chlorine dioxide-based disinfectants tested demonstrated sufficient activity against MDROs meeting the relevant test standards and exhibited similar $\log_{10}$ reductions against organisms within the biofilm model. Sufficient $\log_{10}$ reductions, when tested to the appropriate test standards in realistic contact times against planktonic MDROs, and comparable reductions against biofilms suggest that chlorine dioxide is an attractive candidate for environmental decontamination strategies.

Introduction

Antimicrobial resistance (AMR) is a significant threat to public health, with a prediction that AMR infections will be directly responsible for 10 million deaths per annum, by 2050 [1]. Healthcare-associated infections (HAIs) caused by multidrug-resistant organisms (MDROs) represent a significant challenge to healthcare organizations, with HAIs resulting in poorer patient outcomes and increased treatment cost. Therapeutic options for infections caused by MDROs are limited, resulting in significant morbidity and mortality. The limited development of novel antimicrobials and treatment options for AMR infections underscores the need to prioritize the prevention of infections through effective infection prevention and control (IPC) practices.

A growing concern in healthcare contributing to AMR is the formation of biofilms on surfaces and medical devices. Biofilm is a structured community of micro-organisms that adhere to a surface. Typically, they are embedded within a self-produced matrix known as extracellular polysaccharide substance (EPS). Reduced penetration of antimicrobials via the EPS and the presence of persister cells, which exhibit an altered metabolic state, make biofilms less susceptible to antimicrobials that target active processes [2]. Biofilms can also serve as a reservoir for MDROs and they encourage the transfer for AMR genes [3]. Among all microbial and chronic infections, 65% and

* Corresponding author. Address: Cardiff University, Cardiff, NP20 5DB, UK. Tel.: +44 (0)7841 879295.
E-mail address: norville.phillip@gmail.com (P. Norville).

https://doi.org/10.1016/j.jhin.2025.04.034

80%, respectively, are associated with biofilm formation, highlighting the importance of methods to treat and eradicate them [4].

The healthcare environment is often contaminated with MDROs, many of which exhibit the ability to survive in the environment for extended periods of time [5]. Effective environmental cleaning and disinfection strategies can reduce the bioburden of MDROs in the environment, ultimately reducing the risk of infection transmission. A recent randomized controlled trial demonstrated that increased frequency of cleaning and disinfection of shared medical equipment can reduce the incidence of HAIs by 34% [6]. This highlights the potential impact of effective decontamination strategies on reducing HAI occurrence.

The use of chlorine-releasing agents (CRAs) is a widely practised method for decontaminating healthcare surfaces. Typically, available chlorine is used at a concentration of 1000 parts per million (ppm) for the decontamination of patient rooms when patients have a known or suspected infection. However, there are challenges surrounding the use of some CRAs, including potential damage to medical equipment and occupational health and safety concerns [7].

Chlorine dioxide, a broad-spectrum oxidizing agent, is effective at eliminating bacteria (including most bacterial spores), mycobacteria, viruses, and fungi, providing high-level disinfection of non-porous surfaces, including those of invasive and non-invasive medical devices [8]. Chlorine dioxide is a strong oxidizing agent and, unlike chlorine, does not tend to react with organic materials to form chlorinated species, resulting in a greater ability to kill [9].

Limited data are available on the efficacy of chlorine dioxide and biofilms at in-use concentrations, particularly dry surface biofilms which have been shown to contaminate up to 95% of terminally cleaned items isolated from various UK hospitals [10]. Few models exist to evaluate disinfectant efficacy against developed biofilms, and there is currently no available guidance to substantiate disinfectant effectiveness and claims against biofilm removal in healthcare. The minimum biofilm eradication concentration assay (MBEC) — ASTM E2799 — is one model used to evaluate the initial microbial kill of a disinfectant by introducing a biofilm to the test solution.

Given the role of MDROs and biofilms in exacerbating the AMR crisis, we examined the susceptibility of clinically relevant MDROs, and biofilms formed by clinically relevant pathogens against various chlorine dioxide-based disinfectants using a combination of EN standards and MBEC tests. The standards within EN 14885 cover bactericidal, yeasticidal, fungicidal, virucidal, and sporicidal claims.

Methods

Antimicrobial agents

A variety of different chlorine dioxide solutions (Tristel Solutions, Newmarket, UK) were prepared according to the manufacturer's instructions and tested. Once prepared, all solutions were applied as per the relevant test standard. Sodium hypochlorite Flash (P&G Professional, Weybridge, UK) was used as a positive control at 10,000 parts per million (ppm). The positive control was taken as 10,000 ppm chlorine, as bacterial biofilms can be 1–1000 times more resistant than planktonic cells [11].

EN efficacy testing

EN 14885 is a framework for testing the antimicrobial activity of chemical disinfectants (such as chlorine dioxide). The EN 14885 includes tests for disinfectants to demonstrate microbiocidal effectiveness against bacteria (EN 13727, EN 16615, EN 14561), and fungi (EN 14562). All EN tests conducted as part of this work followed strict adherence to different EN standards (including minimum log reductions, neutralisation, temperature, the presence of interfering (dirty) substance and contact times). Tested solutions have already substantiated biocidal claims according to the EN 14885. In addition to the mandatory organisms stipulated within each standard, other organisms known to be MDROs (Table I) were utilized to examine the efficacy of chlorine dioxide disinfectants under different conditions.

MBEC assay (ASTM E2799)

Preparation of inoculum

Pseudomonas aeruginosa NCIMB 10434 and *Staphylococcus aureus* NCTC 8325 suspensions were prepared in tryptone soya broth (TSB) and serially diluted to produce a final concentration of $1\times10^5 \pm 5\times10^4$ cfu/mL. Suspensions were enumerated by performing dilutions in phosphate-buffered saline (PBS) and plating out the resulting suspensions on to tryptone soya agar (TSA).

Preparation of biofilms

A volume of 150 μL of inoculum was added into separate wells of sterile polystyrene plastic MBEC microtitre 96-well plates for each test agent and control. MBEC plates were then incubated with pegs for 72 h at 37 °C ± 2 °C with agitation at 110 ± 10 rpm, to encourage biofilm formation. Following incubation, the biofilms were rinsed three times in PBS to remove planktonic unadhered bacteria.

Testing of disinfectants

Two hundred microlitres of each test disinfectant were added to specific wells of the microtitre plate. Negative and positive controls were performed concurrently by adding 200 μL of PBS with 1% TSB and 200 μL of sodium hypochlorite (10,000 ppm) solution to specific wells, respectively. Neutralizer, sterility, and growth controls were performed. The MBEC pegs containing the pre-formed biofilms were then immersed into the corresponding wells for 30 s or 5 min treatment time. Following treatment, 200 μL of neutralizer was added to the wells of the microtitre plate and the treated MBEC pegs were immersed. All disinfectants were neutralized with the same neutralizer (Quench (sodium thiosulphate-based)). MBEC plates were placed in a sonicating water bath for 5 min to recover remaining viable organisms. Resulting suspensions were enumerated, serially diluted in PBS, and plated on to TSA and incubated at 37 °C ± 2 °C for 24 h. All testing was performed at $N = 6$ with single technical replicates.

Table I
Test solutions and results of EN efficacy testing of multidrug-resistant organisms

Product	Active substance	Disinfectant type	Test standard	Microorganism	Contact time	Log_{10} reduction
A	ClO_2: 200 ppm	Disinfectant foam	EN 14561	VRE *Enterococcus faecium* NCTC 12204	30 s	>6
				CRE *Klebsiella pneumoniae* NCTC 13443		>6
				ESBL *Klebsiella pneumoniae* ATCC 700603		>5
				MDRAB ATCC BAA-1799		>5
				MRSA NCTC 12493		>5
			EN 16615	MRSA NCTC 12493		>5 Spread: <50 cfu
			EN 13727	MRSA NCTC 12493		>6
			EN 14562	*Candidozyma auris* (formerly *Candida auris*) DM 21092		>5
B	ClO_2: 150 ppm	Liquid solution for wiping	EN 13727	MRSA NCTC 12493	5 min	>5
				ESBL *Klebsiella pneumoniae* ATCC 700603		>5
				MDRAB ATCC BAA-1799		>5
				VRE *Enterococcus faecium* NCTC 12204		>5
				CRE *Klebsiella pneumoniae* NCTC 13443		>5
			EN 14562	*Candidozyma auris* (formerly *Candida auris*) DSM 21092		>4
			EN 16615	*Candidozyma auris* (formerly *Candida auris*) NCPF 8984		>4 Spread: <50 cfu
				Candidozyma auris (formerly *Candida auris*) NCPF 8985		>5 Spread: <50 cfu
C	ClO_2: 120 ppm	Liquid solution for immersing	EN 14561	VRE *Enterococcus faecium* NCTC 12204	5 min	>6
D	ClO_2: 200 ppm	Activated wipe	EN 14561	VRE *Enterococcus faecium* NCTC 12204	30 s	>6
			EN 13727	MRSA NCTC 12493		>5
			EN 14562	*Candidozyma auris* (formerly *Candida auris*) DM 21092		>5
E	ClO_2: 200 ppm	Disinfectant foam	EN 16615	MRSA NCTC 12493	60 s	>5 Spread: <50 cfu
				ESBL *Klebsiella pneumoniae* ATCC 700603		>5 Spread: <50 cfu
				MDRAB NCTC 13420		>5 Spread: <50 cfu
				VRE *Enterococcus faecium* ATCC 6057		>5 Spread: <50 cfu
				CRE *Klebsiella pneumoniae* NCTC 13809		>5 Spread: <50 cfu
				Candidozyma auris (formerly *Candida auris*) NCPF 8984		>4 Spread: <50 cfu
F	ClO_2: 200 ppm and QAC	Disinfectant foam	EN 16615	MRSA NCTC 12493	60 s	>5 Spread: <50 cfu
				ESBL *Klebsiella pneumoniae* NCTC 13465		>5 Spread: <50 cfu
				MDRAB NCTC 13420		>5 Spread: <50 cfu

(*continued on next page*)

Table I (*continued*)

Product	Active substance	Disinfectant type	Test standard	Microorganism	Contact time	Log_{10} reduction
				VRE *Enterococcus faecium* ATCC 6057		>5
						Spread: <50 cfu
				CRE *Klebsiella pneumoniae* NCTC 13809		>5
						Spread: <50 cfu
				Candidozyma auris (formerly *Candida auris*) NCPF 8984		>4
						Spread: <50 cfu
G	ClO_2: 120 ppm	Liquid solution for mopping/wiping	EN 14561	MDRAB NCTC 13420	5 min	>5
			EN 13727	VRE *Enterococcus faecium* NCTC 12204		>5
				CRE *Klebsiella pneumoniae* NCTC 13443		>5
				MRSA NCTC 12493		>6
				MDRAB NCTC 13420		>5
			EN 14562	*Candidozyma auris* (formerly *Candida auris*) DMS 21093		>4

QAC, quaternary ammonium compound; cfu, colony-forming units; MRSA, meticillin-resistant *Staphylococcus aureus*; MDRAB, multidrug-resistant *Acinetobacter baumannii*; CRE, carbapenem-resistant Enterobacterales; VRE, vancomycin-resistant enterococcus; ESBL, extended-spectrum β-lactamase.

Analysis of data and statistical analysis

Data were analysed and visualized using Microsoft Excel. Comparisons of performance of chlorine dioxide efficacy against biofilms were performed using one-way analysis of variance. $P < 0.01$ was considered statistically significant.

Results and discussion

Chlorine dioxide solutions showed microbiocidal efficacy against the panel of MDROs tested, which included Gram-positive and negative bacteria, and a fungal pathogen (Table I). Healthcare surfaces are considered to be a higher risk than general surfaces, containing heavier bioburden and high-risk pathogens. It is important to reduce this bioburden to prevent transmission of pathogens and associated HAIs through fomites. Chlorine dioxide met the criteria for each test, showing a high log_{10} reduction of micro-organisms in all applications within Table I. This data agrees with other studies demonstrating chlorine dioxide's broad efficacy against clinically relevant pathogens [8].

Candidozyma auris (formerly *Candida auris*) is an example of a particularly resistant MDR fungal pathogen. It can rapidly colonize the skin of patients and persist in the healthcare environment for long periods of time, making *C. auris* a challenge for IPC since its emergence in 2009 [12]. It is important that disinfectant manufacturers consider newly emerging pathogens that may not follow the typical hierarchy of resistance. Additionally, manufacturers should ensure that efficacy data encompass a variety of test methodologies, particularly those designed to replicate real-world applications of disinfectants such as EN 14561 and EN 16615.

Most bacteria do not exist in the environment in their planktonic form and would form part of a developed biofilm. Biofilms exhibit increased resistance to antimicrobials, highlighting the importance of assessing the effectiveness of disinfectants against them. All chlorine dioxide disinfectants tested against *S. aureus* and/or *P. aeruginosa* biofilms showed activity at both 30 s and 5 min contact times (Figure 1). The disinfectants consistently achieved >99.99% (4 log_{10}) reductions against both organisms, with greater reductions shown against *P. aeruginosa*. No significant difference ($P \leq 0.01$) was observed when comparing bacterial log_{10} reductions at 30 s and 5 min contact times for both organisms tested.

Details of disinfectants (A–G) are shown in Table I. Error bars represent standard deviation.

High titres of biofilm were visually observed and enumerated from controls (range: 7–8 log_{10}) for both *P. aeruginosa* and *S. aureus* biofilms. The negative control (TSB only) showed a mean log_{10} reduction of 0 against both *P. aeruginosa* and *S. aureus* biofilms. Biofilms of *P. aeruginosa* generally exhibited higher microbial loads compared to those of *S. aureus*, with >4 log_{10} reductions showing a total kill in most cases. Positive controls (10,000 ppm) exhibited mean log_{10} reductions of 6.18 and 4.56 against *P. aeruginosa* and *S. aureus* biofilms, respectively. No significant difference was observed ($P \leq 0.01$) when comparing log_{10} reductions achieved by all chlorine dioxide-based disinfectants (excluding product F, which contains an additional QAC). Chlorine of 10,000 ppm is unsuitable for routine healthcare decontamination due to potential surface damage; however, chlorine dioxide

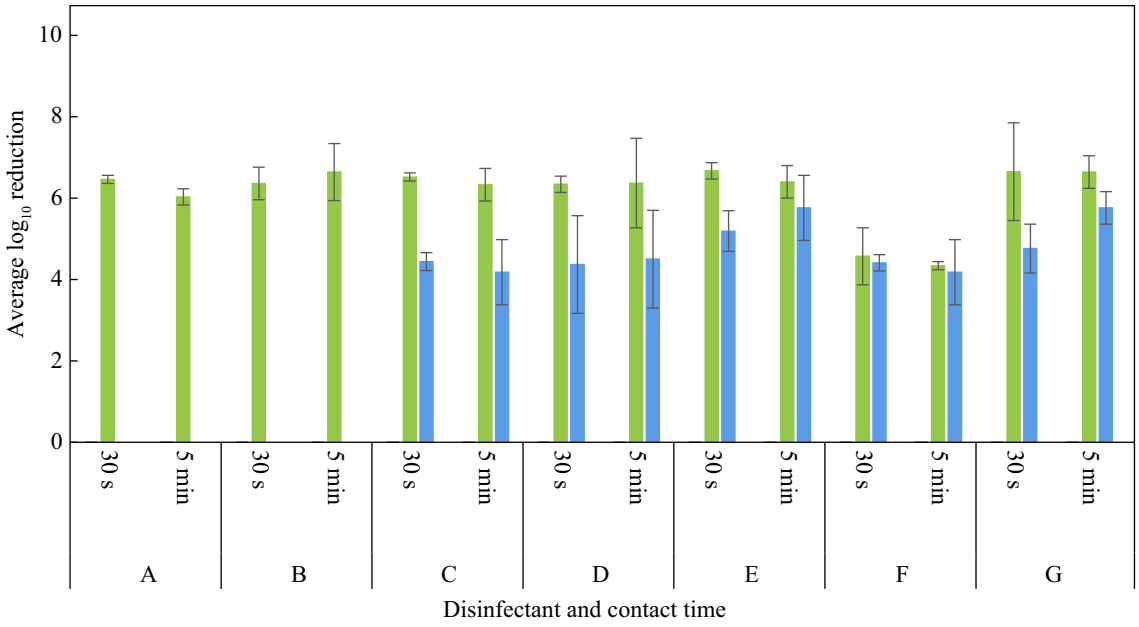

Figure 1. Efficacy of chlorine dioxide disinfectants against biofilms formed by *Pseudomonas aeruginosa* (green bars) and *Staphylococcus aureus* (blue bars).

disinfectants achieved similar efficacy at much lower concentrations (120—200 ppm), making it a suitable contender for IPC decontamination practices [13]. When comparing the efficacy between different concentrations, no significant difference ($P \leq 0.01$) in $\log_{10}$ reduction was observed. Studies have demonstrated chlorine dioxide's effectiveness in removing biofilm in water systems; however, this is the first study demonstrating chlorine dioxide's effectiveness in eliminating bacteria in the MBEC model [14].

There are limitations to this study, principally that testing was conducted using a model developed for screening the efficacy of solutions against biofilm. MBEC is a quick and cost-effective method for first-phase testing of disinfectants; however, efficacy of chlorine dioxide against other biofilm models (such as CDC model) would expand on this study.

Patients admitted to a room where the previous occupant had an MDRO infection results in an increased risk of infection for the next room occupant, highlighting the role of the environment in transmission of HAIs [15]. Prevention of infection reduces the requirement for antimicrobials to be used and may correlate to a reduced risk of AMR developing. It is also appropriate to prevent an infection through proper decontamination practices than to deal with the consequences of treating it later. Given the efficacy of chlorine dioxide against clinically relevant pathogens and the promising results in its effectiveness in eliminating biofilms demonstrated in this study, it stands as an excellent candidate for decontamination practices. Additionally, more guidance is required to support healthcare workers on environmental decontamination practices and products which can support effective killing and removal of bacterial biofilms.

Conflict of interest statement
Dr P. Norville is an independent infection prevention and control consultant for Norvate Limited. S. Hardy and S. Dangleben are employees of Tristel Solutions Limited.

Funding source
All testing was funded by Tristel Solutions Limited.

Ethical approval
Not required.

References

[1] O'Neill J. Review on antimicrobial resistance. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. London: UK Government; 2014.
[2] Maillard JY, Centeleghe I. How biofilm changes our understanding of cleaning and disinfection. Antimicrob Resist Infect Control 2023;12:95.
[3] Bowler P, Murphy C, Wolcott R. Biofilm exacerbates antibiotic resistance: is this a current oversight in antimicrobial stewardship? Antimicrob Resist Infect Control 2020;9:162.
[4] Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, et al. Bacterial biofilm and associated infections. J Chin Med Assoc 2018;81:7—11.
[5] Kramer A, Lexow F, Bludau A, Köster AM, Misailovski M, Seifert U, et al. How long do bacteria, fungi, protozoa, and viruses retain their replication capacity on inanimate surfaces? A systematic review examining environmental resilience versus healthcare-associated infection risk by 'fomite-borne risk assessment'. Clin Microbiol Rev 2024;37(4):e0018623.
[6] Browne K, White N, Tehan P, Russo PL, Amin M, Stewardson AJ, et al. A randomised controlled trial investigating the effect of improving the cleaning and disinfection of shared medical equipment on healthcare-associated infections: the CLEaning and Enhanced disiNfection (CLEEN) study. Trials 2023;24:133.
[7] Chung I, Ryu H, Yoon SY, Ha JC. Health effects of sodium hypochlorite: review of published case reports. Environ Anal Health Toxicol 2022;37(1):e2022006-0.
[8] Westerway SC, Basseal JM, Abramowicz J, Moran C. Recommendations for the cleaning of endocavity ultrasound transducers between patients. Ultrasound Med Biol 2024;50:775—8.

[9] Andrés CMC, Lastra JMP, Andrés Juan C, Plou FJ, Pérez-Lebeña E. Chlorine dioxide: friend or foe for cell biomolecules? A chemical approach. Int J Mol Sci 2022;23(24):15660.
[10] Ledwoch K, Dancer SJ, Otter JA, Kerr K, Roposte D, Rushton L, et al. Beware biofilm! Dry biofilms containing bacterial pathogens on multiple healthcare surfaces; a multi-centre study. J Hosp Infect 2018;100:E47—56.
[11] Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F. Resistance of bacterial biofilms to disinfectants: a review. Biofouling 2011;27:1017—32.
[12] Welsh RM, Bentz ML, Shams A, Houston H, Lyons A, Rose LJ, et al. Survival, persistence, and isolation of the emerging multidrug-resistant pathogenic yeast Candida auris on a plastic health care surface. J Clin Microbiol 2017;55:2996—3005.
[13] Dettenkofer M, Block C. Hospital disinfection: efficacy and safety issues. Curr Opin Infect Dis 2005;18:320—5.
[14] Bova G, Hopkins J, Sharpe P, Keane T. Evaluation of chlorine dioxide in potable water systems for legionella control in an acute care hospital environment. In: Presented at the 65th annual international water conference; 2004. Pittsburgh, PA, USA, October 17th—21st.
[15] Mitchell BG, McDonagh J, Dancer SJ, Ford S, Sim J, Thottiyil Sultanmuhammed Abdul Khadar B, et al. Risk of organism acquisition from prior room occupants: an updated systematic review. Infect Dis Health 2023;28:290—7.