Journal of Hospital Infection 162 (2025) 232–240

Available online at www.sciencedirect.com

Journal of Hospital Infection

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

Exploring the bactericidal efficacy of a new potassium monopersulphate-based disinfectant

P.P. Barbosa [a,b], D.M. Leme [d], N.G. Motta [c], W.L.E. Magalhães [c,d], J.L. Proenca-Modena [b], J-Y. Maillard [a,*]

[a] School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, Wales, UK
[b] University of Campinas (UNICAMP), Campinas, São Paulo, Brazil
[c] Embrapa Florestas, Paraná, Brazil
[d] Department of Genetics, Federal University of Paraná (UFPR), Curitiba, Paraná, Brazil

ARTICLE INFO

Article history:
Received 17 February 2025
Accepted 23 April 2025
Available online 12 May 2025

Keywords:
Disinfectant
Efficacy
Bacteria
Suspension test
Dry surface biofilm

SUMMARY

Background: *Staphylococcus aureus* and *Klebsiella pneumoniae* are common pathogens responsible for hospital-acquired infections. Both species can survive on surfaces following desiccation and form dry surface biofilms (DSBs), which complicates the disinfection process.
Aim: To evaluate the efficacy of an innovative potassium monopersulphate-based nano-technology formulation (MPS) against both planktonic and sessile *S. aureus* and *K. pneumoniae*.
Methods: The bactericidal efficacy of MPS was tested in comparison with sodium hypochlorite (NaOCl) and didecyldimethylammonium chloride (DDAC), which served as controls. The assessment was performed against planktonic bacteria, hydrated biofilm, and DSB using standard suspension and carrier tests. Scanning electron microscopy (SEM) was employed to identify any gross structural damage.
Findings: MPS (2% w/v) achieved a $\geq$4 $\log_{10}$ reduction in *K. pneumoniae* with a short contact time, regardless of the test protocol. *S. aureus* was more resilient, but the introduction of wiping reduced the contact time needed to achieve a 4 $\log_{10}$ reduction from 15 to 5 min. SEM analysis revealed gross structural damage in both species following MPS treatment. The other disinfectants tested were also bactericidal, achieving $\geq$4 $\log_{10}$ reduction within 1–5 min, with the exception of DDAC against hydrated biofilms.
Conclusion: The potassium monopersulphate-based formulation was found to be an effective bactericide, including against DSBs. Its efficacy compares favourably with other biocides commonly used in healthcare settings, and its biodegradability makes it a promising candidate for further development. However, optimization of the mechanical removal process will be essential to enhance MPS efficacy in practical applications.

* Corresponding author. Address: School of Pharmacy & Pharmaceutical Sciences, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3NB, UK. Tel.: +44 (0)2920 879088.
E-mail address: maillardj@cardiff.ac.uk (J-Y. Maillard).

https://doi.org/10.1016/j.jhin.2025.04.033

Introduction

Cleaning and disinfection are two of the most important factors in controlling the spread of pathogens and reducing healthcare-associated infections (HAIs), as some micro-organisms can survive for months on dry surfaces [1]. Formulated biocides, therefore, play a crucial role as disinfectants by controlling micro-organisms present on contaminated surfaces, reducing the number of pathogens, and consequently their transmission [2].

New biocidal formulations should undergo rigorous antimicrobial efficacy testing following appropriate protocols to ensure that the product will work in practice [3]. Indeed, the performance of disinfectants varies depending on the test bacteria, test conditions (planktonic, dried on a surface, sessile in a hydrated biofilm, or DSB), and contact time [2]. The types of antimicrobial tests conducted, as well as factors relevant to product usage — such as soiling, contact time, surface type, and target micro-organism — should all be considered [4]. For some biocidal products, this may include wiping, which can contribute to the overall product efficacy [5].

Staphylococcus aureus and *Klebsiella pneumoniae* are common pathogens associated with surfaces and materials in healthcare settings, particularly intensive care units (ICUs) [6]. These pathogens are responsible for HAIs and are linked with comorbidity in vulnerable patients [7—9]. These bacteria can also survive on surfaces after desiccation and form dry surface biofilms (DSBs), making the disinfection process more challenging [10,11].

This study aims to evaluate the bactericidal efficacy of an innovative potassium monopersulphate-based nanotechnology formulation, designed as a disinfectant with controlled release of the active compound. This formulation was developed during the COVID-19 pandemic for large-scale virucidal disinfection in healthcare settings, homes, and public spaces. It explores the impact of test protocols and parameters on efficacy, with the goal of understanding the limitations of this new formulation and assessing its potential as a broad-spectrum disinfectant.

Methods

Bacterial growth

S. aureus NCTC 10788 and *K. pneumoniae* ATCC 13883 were prepared from −80 °C stock in 20 mL of sterile tryptone soya broth (TSB; Oxoid Ltd, Basingstoke, UK) and incubated aerobically overnight at 37 °C with shaking at 120 rpm in a Sanyo orbital shaker. After 24 h of incubation, the bacterial suspensions were centrifuged at 5000 *g* for 10 min, and the pellets were resuspended in 10 mL of sterile TSB. The washed bacterial suspension contained $\sim 10^6$ cfu/mL and was used as the test inoculum.

Hydrated biofilm sedimentation

Hydrated sedimentation biofilms were developed as described by Duggan *et al.* [12]. The washed test inocula were diluted 100-fold in additional TSB, then supplemented with bovine serum albumin (BSA) to a final concentration of 0.3 g/L. One millilitre of this suspension was added to each well of a 24-well plate containing one sterile stainless-steel disc, and the plate was incubated for 96 h at 21 °C with agitation (150 rpm in a microplate shaking platform). The medium was then removed, and the discs were gently washed with tryptone sodium chloride (TSC). Treatments and subsequent steps were carried out as outlined above.

Dry surface biofilm preparation

DSB preparation followed the protocol described by Ledwoch *et al.* [13]. DSBs of each bacterial species were formed on sterile stainless-steel discs placed at the bottom of each well in a 24-well culture plate. One millilitre of the washed test inoculum ($\sim 1 \times 10^6$ cfu/mL), supplemented with 3 g/L BSA, was added to each well (initial wet phase), and the plates were incubated with continuous shaking (150 rpm in a microplate shaking platform) at room temperature (20—23 °C) for 48 h. The medium was then drained, and the plate was incubated for 48 h in an incubator at 37 °C (dry phase). Alternating wet and dry phases were performed for 12 cycles to form a mature DSB. DSB treatments and subsequent steps were performed as outlined above.

Biocides tested

A novel potassium monopersulphate (Oxone®-based) nanotechnology formulation (MPS), containing 2% w/v potassium monopersulphate (Oxone®, Sigma), 0.6% w/v cellulose nanofibres, and 0.8% w/v chitosan nanoparticles, was developed by the Brazilian Agricultural Research Corporation (EMBRAPA) in collaboration with the Federal University of Paraná (UFPR, Brazil). For comparison, this study also included two disinfectants commonly used in healthcare settings: 2% w/v sodium hypochlorite (NaOCl; 20,000 ppm) and 2% w/v didecyldimethylammonium chloride (DDAC), a quaternary ammonium compound (QAC).

Neutralizer efficacy and toxicity test

Before conducting the disinfectant tests, efficacy tests were performed with both bacteria to verify whether the selected neutralizer was effective in halting the action of the disinfectant (neutralizer efficacy test). Additionally, a toxicity test was performed to ensure that the neutralizer was not toxic to the bacteria. Both tests followed the protocol of Messager *et al.*, with a modification to the contact time (30 min) [14]. The 'universal' neutralizer used contained saponin (30 g/L), L-histidine (1 g/L), polysorbate-80 (30 g/L), sodium thiosulphate (3 g/L), L-α-lecithin (3 g/L), sodium chloride (8.5 g/L), and tryptone (1 g/L) in distilled water.

Testing for microbicidal efficacy

Several microbicidal test protocols were conducted: suspension test (in Europe, equivalent to phase 2 step 1 standard tests), carrier tests (equivalent to phase 2 step 2 tests), and then product test combining efficacy with mechanical removal (also equivalent to phase 2 step 2 tests). Bacterial susceptibility to antimicrobials decreases from planktonic (suspension

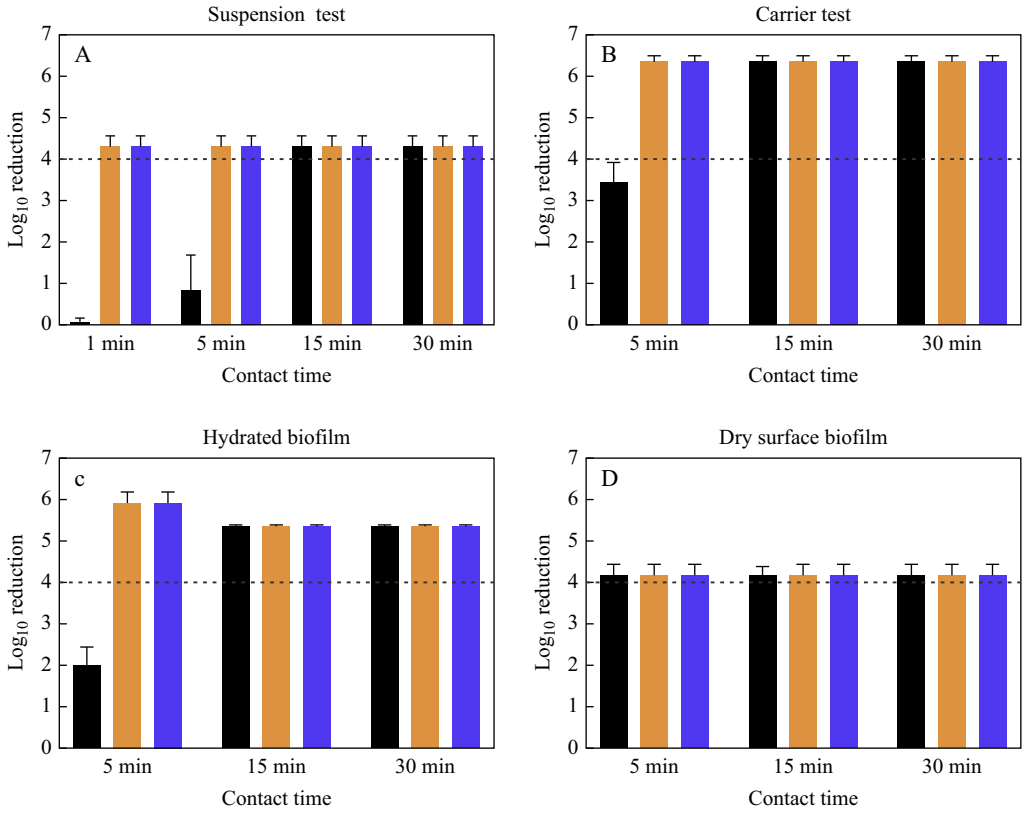

Figure 1. Log_{10} reduction in *S. aureus* after treatment with potassium monopersulphate (MPS), sodium hypochlorite (NaOCl) and didecyldimethylammonium chloride (DDAC). (A) Suspension test, (B) carrier test, (C) hydrated biofilm, (D) dry surface biofilm. All log_{10} reduction values were calculated relative to the log_{10} value of the control (with TSC); $N = 3$. Black bars: MPS; orange bars: NaOCl (2% w/v); blue bars: DDAC (2% w/v).

test), to dried on surfaces (carrier test), with bacteria in biofilms being the least susceptible [15].

Suspension test

Suspension test was performed according to BS EN 13704:2018 with some modifications as follows [16]. Thirty microlitres of washed bacterial inoculum (10^6 cfu/mL), 30 μL of TSC supplemented with 3 g/L of bovine serum albumin (BSA), and 240 μL of the disinfectant were mixed in an Eppendorf tube (1:1:8 ratio) at room temperature (20—23 °C) for contact times of 1, 5, 15, and 30 min. Controls consisted of using TSC (8.5 g/L NaCl, 1 g/L tryptone) instead of the disinfectant.

After the appropriate contact time (1, 5, 15 or 30 min), 100 μL of the mixture was transferred to 900 μL of a universal neutralizer. The drop counting method was then used to enumerate bacterial survivors [17].

Hard surface carrier test

Hard surface carrier test was conducted according to BS EN 13697:2015+A1:2019 [18]. Twenty-five microlitres of washed bacterial inoculum (10^6 cfu/mL) were deposited onto sterile stainless-steel discs (AISI 430; 0.7 ± 0.07 mm thickness; 10 ± 0.5 mm diameter) and allowed to dry for 1 h in a biosafety level 2 cabinet. After treatment with the disinfectants, the discs were transferred to McCartney bottles containing 3 g of glass beads and 2 mL of universal neutralizer. The samples were then vortexed for 4 min, and a 10-fold dilution series was performed in sterile TSC. Log_{10} reduction values were calculated relative to the controls (untreated discs).

'Product test' combining mechanical removal and disinfection

To explore how to reduce contact time, the efficacy of combining mechanical removal and disinfectants was tested based on the ASTM E2967-15 standard [19]. A dried inoculum on stainless-steel discs was prepared as described above. The discs were exposed to a 5 min contact time with the disinfectants and then wiped with a 2 cm × 2 cm square piece of all-purpose cleaning cloth (80% polyester and 20% polyamide) attached to the Wiperator (wiping time: 15 s; weight: 300 g [19]. Prior to use, the cloth pieces were exposed to UV-C light (in a biosafety cabinet) for 20 min on each side. After wiping, the discs were transferred to McCartney bottles containing 2 mL of universal neutralizer and processed as described above. As a control, discs were treated with distilled water for 5 min, followed by wiping with the Wiperator using the same parameters mentioned above.

The ASTM E2967-15 standard also measures the bacterial transferability of a contaminated cloth to a new surface [19]. Contaminated discs were wiped as described above, but the contaminated cloth was then used to wipe a sterile disc (wiping time: 15 s; weight: 300 g). The wiped discs were then transferred to a McCartney bottle containing 2 mL of universal neutralizer and processed as described above.

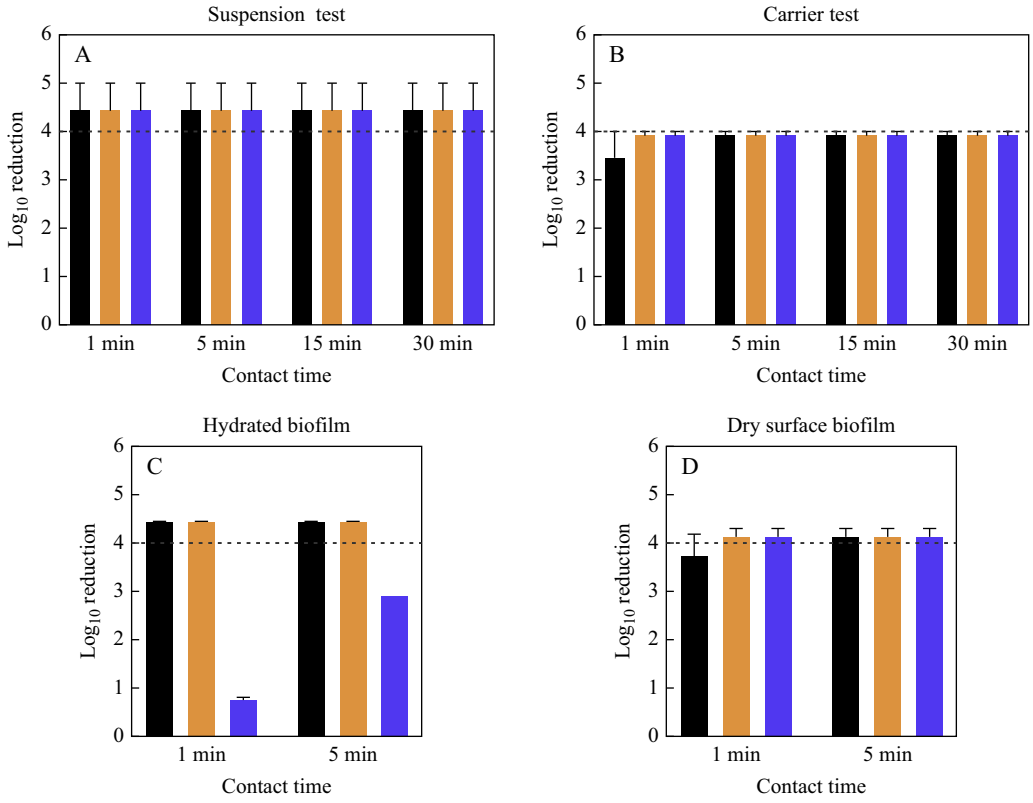

Figure 2. Log_{10} reduction in *K. pneumoniae* after treatment with potassium monopersulphate (MPS), sodium hypochlorite (NaOCl) and didecyldimethylammonium chloride (DDAC). (A) Suspension test, (B) carrier test, (C) hydrated biofilm, (D) dry surface biofilm (DSB). All log_{10} reduction values were calculated relative to the log_{10} value of the control (with TSC); $N = 3$. Black bars: MPS; orange bars: NaOCl (2% w/v); blue bars: DDAC (2% w/v).

Determination of efficacy

The efficacy of each treatment against planktonic bacteria in suspension, planktonic bacteria dried on surfaces (with or without wiping), as well as against hydrated biofilm and DSB, was evaluated by measuring the log_{10} reduction in cfu/mL. This was calculated as the log_{10} difference in cfu between control tests using TSC and tests using disinfectants.

Scanning electron microscopy (SEM)

High-vacuum SEM was used to visualize the DSB structure, either following a disinfectant treatment or TSC as a control. Control and post-treatment DSB samples were prepared by overnight incubation of discs in a 2.5% glutaraldehyde solution, followed by a series of 10 min ethanol washes in increasing concentrations (5%, 25%, 50%, 75%, 90%, and 100%). The samples were then coated with a thin layer (20 nm) of gold–palladium using a Bio-Rad Sputter Coater SC500, following an argon purge of the sputter chamber. The InLens setting on a Philips XL30 field emission gun-scanning electron microscope (FEG-SEM) was used to capture images at $\times$10,000 and $\times$5000 magnification, with a working distance of 5–7 mm.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9.2.0 (version 9.3.1), with log_{10} reduction values analysed using two-way analysis of variance (ANOVA). All measurements included a minimum of three biological replicates.

Results

Neutralizer efficacy and toxicity tests

After conducting efficacy and toxicity tests, the universal neutralizer exhibited no toxicity to the bacteria and effectively neutralized the disinfectants. In all experimental triplicates, a reduction of <0.2 log_{10} in bacterial concentration was observed (data not shown). This result was not significantly different from the control samples, which contained neither the neutralizer nor the disinfectant.

Impact of the test methods on efficacy

A 4 log_{10} reduction in bacterial viability is required to pass EN standard tests, such as BS EN 13697:2015+A1:2019 [18]. In this study, we used a 4 log_{10} reduction as a benchmark for efficacy to compare the effectiveness of the different disinfectants tested under various protocols.

The different test methods used in this study did not affect the efficacy of NaOCl (20,000 ppm) or DDAC (2% w/v) against *S. aureus*, both achieving >4 log_{10} reduction within 5 min, including against DSB (Figure 1). The 2% w/v potassium monopersulphate (Oxone-based) formulation achieved a >4 log_{10}

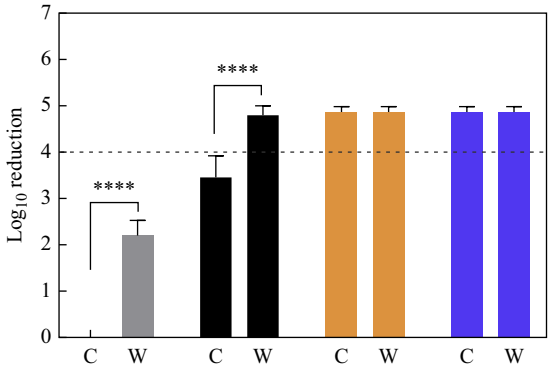

Figure 3. Reduction in *S. aureus* viability by combining wiping and disinfectants. C (Control) for treated discs without the use of the Wiperator; W (Wiped) for discs treated and then wiped with the Wiperator. All $\log_{10}$ reduction values were calculated relative to the $\log_{10}$ value of the water control (C); $N = 3$. ****$P \leq 0.0001$; two-way analysis of variance. Grey bars: water; black bars: potassium monopersulphate (MPS); orange bars: sodium hypochlorite (NaOCl) (2% w/v); blue bars: didecyldimethylammonium chloride (DDAC) (2% w/v).

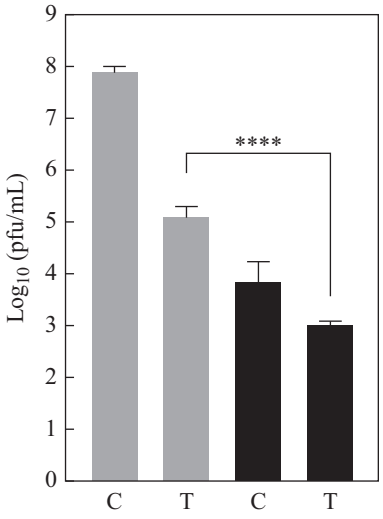

Figure 4. Transfer of *S. aureus* following a combination of wiping and disinfection. C (Control) for treated discs without the use of the Wiperator; T (Transferred) for sterile discs wiped with the same cloth used on the treated discs with potassium monopersulphate (MPS); $N = 3$. ****$P \leq 0.0001$; two-way analysis of variance. Grey bars: water; black bars: MPS.

reduction only after 15 min, regardless of the test protocol (Figure 1). However, shorter contact times (5 min) significantly impacted the efficacy of MPS, with $\log_{10}$ reductions ranging from 0.05 to 4.4. Interestingly, this formulation performed better when bacteria were dried on surfaces or in DSB (Figure 1B, D).

With *K. pneumoniae*, all disinfectants achieved a >4 $\log_{10}$ reduction within 1 min of contact time in the suspension test (Figure 2A). Both DDAC (2% w/v) and NaOCl (2% w/v) also achieved a 4 $\log_{10}$ reduction within 1 min in the carrier test and against DSB (Figure 2B, D). However, DDAC killed <3 $\log_{10}$ of *K. pneumoniae* in hydrated biofilm, even after 5 min of contact. Within 5 min, the MPS formulation also achieved a 4 $\log_{10}$ reduction when tested against biofilms (Figure 2C, D). At a 5 min contact time, there was no significant difference in efficacy between MPS and NaOCl (20,000 ppm) (two-way ANOVA).

Disinfectants are typically used on surfaces in combination with a cloth material or wipe [5]. Therefore, combining disinfectant efficacy with mechanical removal can enhance product performance. In this study, we tested the disinfectants against *S. aureus* dried on surfaces, with and without wiping, as the results with the Oxone-based formulation did not achieve a 4 $\log_{10}$ reduction within 5 min (Figure 1B).

The additional mechanical removal (15 s wiping time; weight: 300 g) in combination with MPS increased the efficacy of the product to >4 $\log_{10}$ reduction in 5 min (Figure 3). For NaOCl and DDAC, no further efficacy was observed with the addition of wiping, as the limit of detection was reached (≈ 5 $\log_{10}$ reduction) (Figure 3). Of note, wiping with the cloth material and water produced a 2.5 $\log_{10}$ *S. aureus* removal from the disc surface.

The ASTM E2967-15 test also measures microbial transfer from the wipe after wiping [19]. In this study, we examined bacterial transfer from the wipe with and without wiping (15 s wiping time; 300 g weight). Without wiping, MPS (2% w/v) significantly reduced bacterial transfer from the wipe ($P \leq 0.0001$; two-way ANOVA; Figure 4). After wiping, the decrease in *S. aureus* transfer from the wipe to a sterile surface was significantly greater ($P \leq 0.0001$; two-way ANOVA) when wiping was combined with the Oxone-based formulation, compared to using water alone. The bacterial transfer decreased from 5 $\log_{10}$ to ~ 3 $\log_{10}$ (Figure 4).

To explore the mechanisms of bactericidal action of the Oxone-based formulation against bacteria in DSB, SEM was performed to identify any gross morphological damage. Untreated *S. aureus* cells appeared smooth (Figure 5A; $\times 1000$ magnification), although at higher magnification, some cracks were visible (Figure 5A; $\times 10{,}000$ magnification). Both MPS (2% w/v) and NaOCl (20,000 ppm) caused the release of some cellular material, and cells showed signs of blebbing (Figure 5B, C). Exposure to DDAC (2% w/v) did not appear to impact cell morphology (Figure 5D). By contrast, *K. pneumoniae* cells in DSB appeared dehydrated, but their surface remained smooth despite visible cracks (Figure 6A). Exposure to the Oxone-based formulation and NaOCl (20,000 ppm) resulted in the formation of blebs on the surface (Figure 6B, C), although these were less pronounced compared to *S. aureus*.

The effect of DDAC (2% w/v) against *K. pneumoniae* cells in DSB also resulted in the presence of blebs but to a much lesser effect compared to the other two disinfectants, as suggested by the obtained images (Figure 6D). Yet the release of materials from *S. aureus* following exposure to MPS (2% w/v) and NaOCl (20,000 ppm), evident at low magnification (Figure 5B, C), was not evident from *K. pneumoniae* (Figure 6B, C).

Discussion

The primary objective of this study was to assess the efficacy of a newly developed disinfectant containing nanomaterials that control the release of the active compound, which consists of 2% potassium monopersulphate (Oxone), against two bacterial pathogens, under various test conditions and contact times. Our results showed that the MPS (2% w/v)

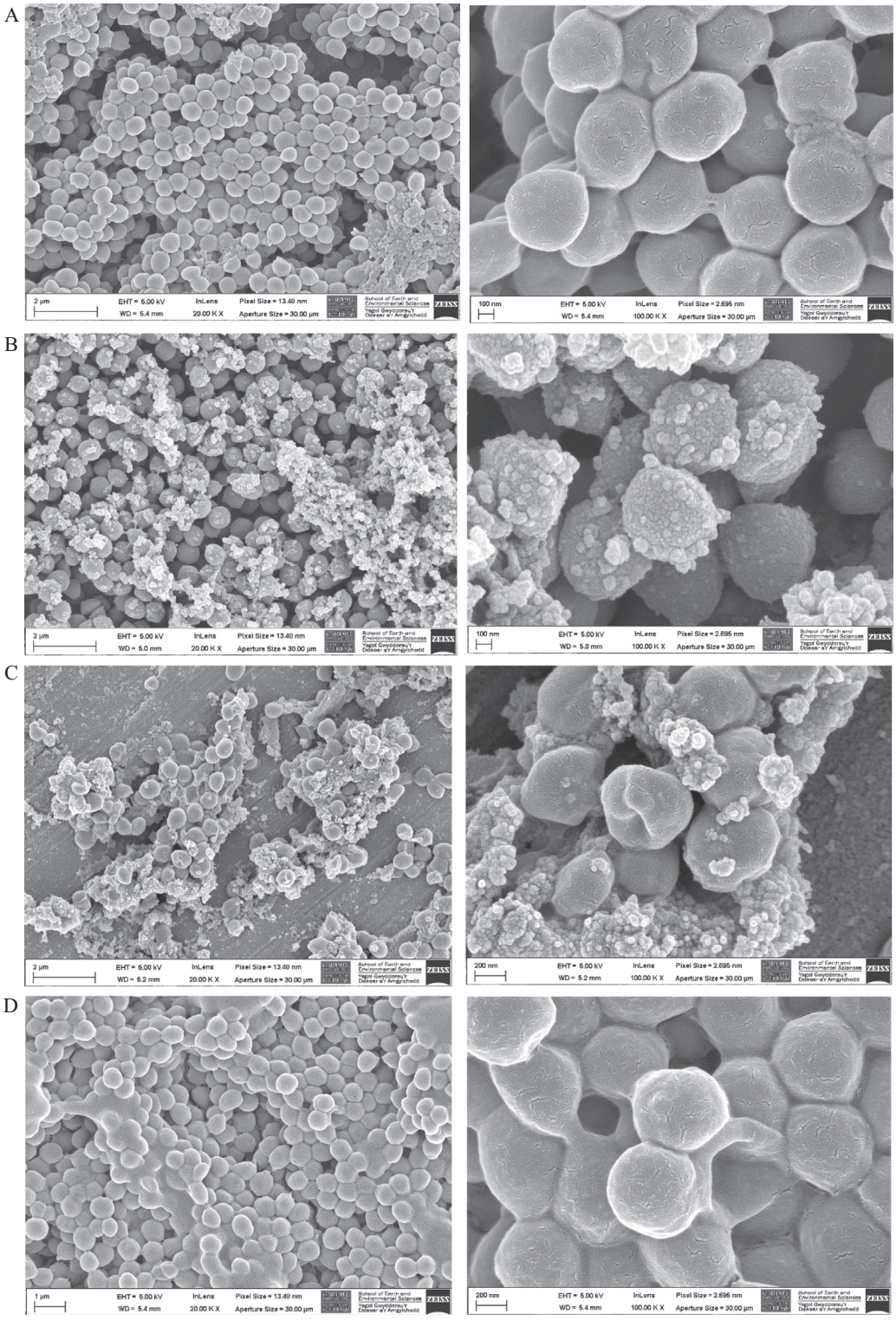

Figure 5. Scanning electron microscope imaging of *S. aureus* dry surface biofilms (DSBs) samples (on stainless steels discs) at ×1000 magnification (*left*) and ×10,000 magnification (*right*). (A) Control; (B) treated with potassium monopersulphate (2% w/v); (C) treated with sodium hypochlorite (20,000 ppm); (D) treated with didecyldimethylammonium chloride (2% w/v). DSBs were exposed to a 30 min contact time for each product tested.

formulation resulted in a 4 $\log_{10}$ reduction in 1 min against *K. pneumoniae* but needed a 15 min contact time against *S. aureus* to achieve the same efficacy. The combination of MPS with mechanical removal (15 s wiping) produced a 4 $\log_{10}$ reduction in 5 min with *S. aureus*. The lack of efficacy of MPS at shorter contact time (<5 min) without mechanical removal could be imparted to the main active that might be less reactive than the oxidizing agent (NaOCl), although further data

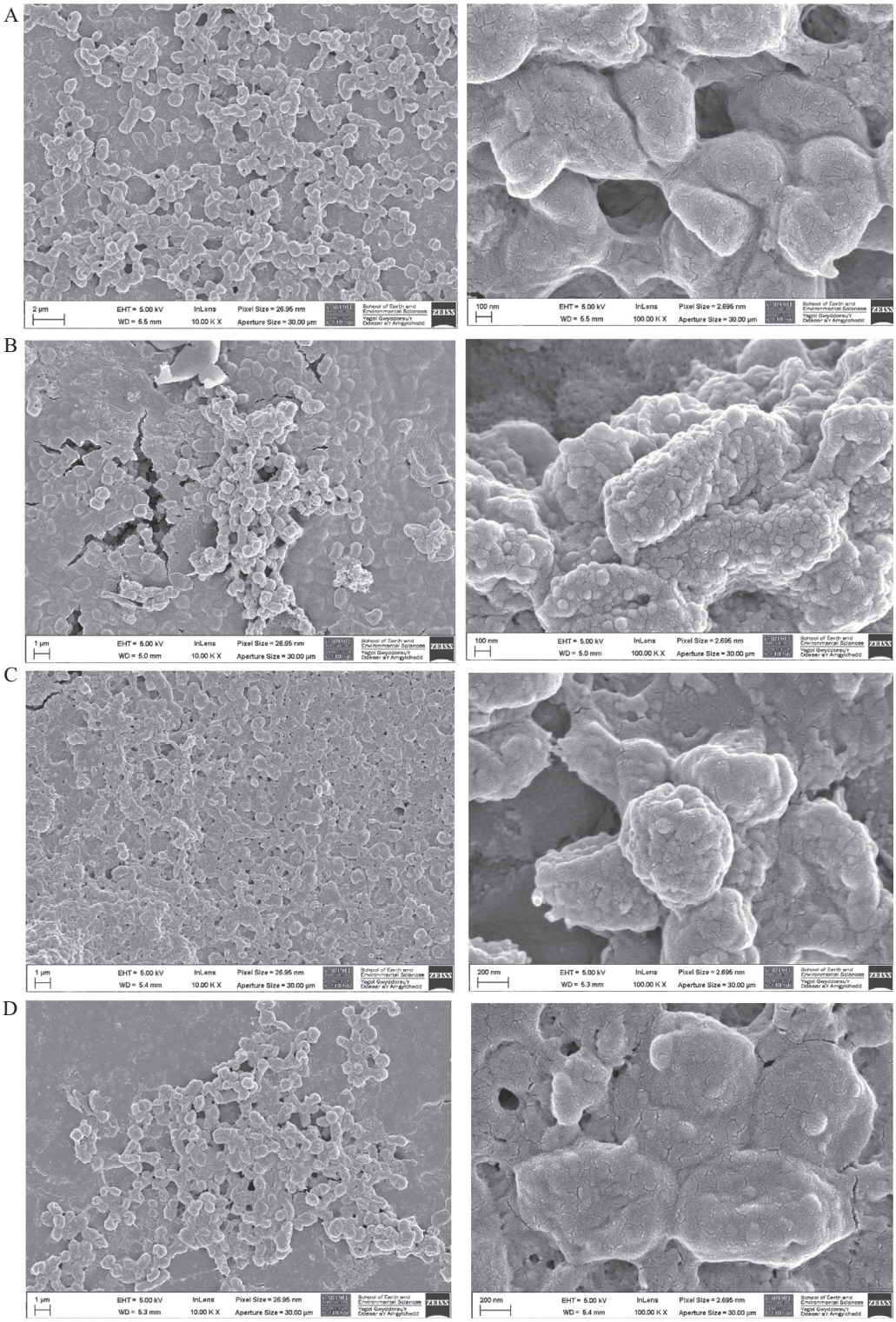

Figure 6. Scanning electron microscope imaging of *K. pneumoniae* dry surface biofilms (DSBs) samples (on stainless steels discs) at ×1000 magnification (left) and ×10,000 magnification (right). (A) Control; (B) treated with potassium monopersulphate (2% w/v); (C) treated with sodium hypochlorite (20,000 ppm); (D) treated with didecyldimethylammonium chloride (2% w/v). DSBs were exposed to a 30 min contact time for each product tested.

including inactivation kinetic using shorter contact times combined with membrane damage monitoring such as using Syto-9/propidium iodide fluorescent markers or potassium release would be valuable [20,21].

Here the test protocols did not impact greatly on the efficacy of the biocides used, probably because of the high concentration used, notably with NaOCl, although other authors highlighted the impact of test protocols on efficacy when

evaluating disinfectants [22,23]. The combination of mechanical removal and MPS significantly improved the formulation's efficacy, particularly against *S. aureus*. The efficiency of mechanical removal of micro-organisms through wiping, as observed in our results, has been well-documented in the literature for both viruses and bacteria [24–26].

The bacterial species selected for this study exhibited significantly different responses to the contact times with MPS (2% w/v). For *S. aureus*, achieving a $\geq$4 $\log_{10}$ reduction required a longer contact time (between 5 and 15 min) compared to *K. pneumoniae* (between 30 s and 1 min) (Figures 1 and 2).

NaOCl (20,000 ppm) and DDAC (2% w/v) achieved more than a 4 $\log_{10}$ reduction under all tested conditions. We acknowledge that the concentration of NaOCl used here was higher than those typically used in healthcare settings (usually 1000–10,000 ppm), which likely contributed to its superior efficacy. Compared to MPS (2% w/v), these compounds required a shorter contact time to achieve this level of efficacy. However, when considering factors such as toxicity and sustainability, the Oxone-based disinfectant may offer advantages. Elevated concentrations of hypochlorite in water and soil can be toxic to plants and animals and pose inhalation and ingestion risks to humans [27].

Additionally, the increased use of quaternary ammonium compounds and their higher concentrations in wastewater pose potential risks to aquatic life and contribute to the development of antimicrobial resistance [28]. By contrast, Oxone is regarded as an environmentally friendly oxidizing agent, making it suitable for use in disinfection applications [29,30]. In this study, MPS was formulated with 2% Oxone and supplemented with $<$1% of nanomaterials. Specifically, cellulose nanofibres were incorporated to improve surface adhesion, while chitosan nanoparticles were used to control the release of the active compound, both of which are documented for their biodegradability and have garnered significant attention in the development of sustainable products [31–33].

There is a notable lack of testing on the efficacy of disinfectants against DSBs, which are commonly found in hospitals, even with regular cleaning and disinfectant use, posing a potential risk of contamination to patients and healthcare workers [34]. Our study evaluated the efficacy of MPS against DSBs and demonstrated that the bacteria in DSBs were effectively killed after treatment. However, one limitation of our study is that, although we achieved a 4 $\log_{10}$ reduction and observed gross damage with SEM, we did not analyse the potential for bacterial biofilm regrowth after treatment. This is a concern, as reported by Almatroudi *et al.*, who found that live *S. aureus* cells remained and regrew even after 10 min of treatment with sodium hypochlorite [10]. Additionally, our results demonstrated that the bacteria transferred by the control (treated with water) were significantly higher than in samples treated with disinfectants. This underscores the importance of using disinfectants to prevent the transfer of bacteria from one high-touch surface to another, thereby reducing the risk of transmission. This is particularly critical in hospital environments, where healthcare workers frequently touch contaminated surfaces and can carry micro-organisms on their gloves, potentially transferring these pathogens to vulnerable patients [35]. Daily disinfection of high-touch surfaces can significantly reduce the transmission of pathogens in hospitals [36].

In conclusion, our study highlighted several limitations of the potassium monopersulphate-based formulation, particularly the need to combine it with mechanical removal to achieve a 4 $\log_{10}$ reduction in bacterial concentration. However, it demonstrated activity against bacteria in both hydrated and dry biofilms, which are typically more difficult to eliminate. Overall, our data on the efficacy of MPS compared favourably with other disinfectants commonly used in healthcare settings.

Acknowledgements

Special thanks to Dr D. Muir (Cardiff University) for providing the SEM images, and to Dr W. Marciel de Souza (University of Kentucky) for reviewing this paper and providing support throughout the project.

Conflict of interest statement

None declared.

Funding sources

Funding for study design and provision of materials for this study was provided by Cardiff University. Disinfectant development was financed by CAPES (Epidemics Call for Proposals 09/2020 Grant No. 673/2020) and FAPESP (process number 2016/00194–8 and 2020/04558–0). P.P. Barbosa was funded by CAPES (process number 88887.505138/2020–00, 88887.513267/2020–00 and 88887.937067/2024–00). J.L. Proenca-Modena was funded by CNPq (process number 309971/2023–3).

Ethical approval

Not required.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGTP in order to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and they take full responsibility for the content of the publication.

References

[1] Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis 2006;6:130.
[2] Maillard J-Y, Pascoe M. Disinfectants and antiseptics: mechanisms of action and resistance. Nature Rev Microbiol 2024;22:4–17.
[3] Maillard J-Y. Testing the effectiveness of disinfectants and sanitisers. In: Holah J, Lelieveld HLM, Gabric D, editors. Handbook of hygiene control in the food industry. 2nd edn. Amsterdam: Elsevier; 2005. p. 641–71.
[4] Artasensi A, Mazzotta S, Fumagalli L. Back to basics: choosing the appropriate surface disinfectant. Antibiotics 2021;10:613.
[5] Sattar SA, Maillard J-Y. The crucial role of wiping in decontamination of high-touch environmental surfaces: review of current status and directions for the future. Am J Infect Control 2013;41:S97–104.
[6] Dancer SJ. Importance of the environment in meticillin-resistant *Staphylococcus aureus* acquisition: the case for hospital cleaning. Lancet Infect Dis 2008;8:101–13.
[7] Cheung GYC, Bae JS, Otto M. Pathogenicity and virulence of *Staphylococcus aureus*. Virulence 2021;12:547–69.
[8] Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE. A genomic surveillance framework and genotyping tool for

Klebsiella pneumoniae and its related species complex. Nature Com 2021;12:4188.
[9] Samuel P, Kumar YS, Suthakar BJ, Karawita J, Kumar DS, Vedha V, et al. Methicillin-resistant *Staphylococcus aureus* colonization in intensive care and burn units: a narrative review. Cureus 2023;15:e47139.
[10] Almatroudi A, Altahir S, Hu H, Chowdhury D, Gosbell IB, Jensen SO, et al. *Staphylococcus aureus* dry-surface biofilms are more resistant to heat treatment than traditional hydrated biofilms. J Hosp Infect 2018;98:161—7.
[11] Centeleghe I, Norville P, Hughes L, Maillard J-Y. *Klebsiella pneumoniae* survives on surfaces as a dry biofilm. Am J Infect Control 2023;51:1157—62.
[12] Duggan K, Shepherd M, Maillard J. Susceptibility of *Salmonella enterica* Typhimurium dry surface biofilms to disinfection. J Food Safety 2024;44:e13117.
[13] Ledwoch K, Said J, Norville P, Maillard J-Y. Artificial dry surface biofilm models for testing the efficacy of cleaning and disinfection. Lett Appl Microbiol 2019;68:329—36.
[14] Messager S, Goddard PA, Dettmar PW, Maillard J-Y. Determination of the antibacterial efficacy of several antiseptics tested on skin by an 'ex-vivo' test. J Med Microbiol 2001;50:284—92.
[15] Otter JA, Vickery K, Walker JT, deLancey Pulcini E, Stoodley P, Goldenberg SD, et al. Surface-attached cells, biofilms and biocide susceptibility: implications for hospital cleaning and disinfection. J Hosp Infect 2015;89:16—27.
[16] BS EN13704:2018. Chemical disinfectants. Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements (phase 2, step 1). London: British Standards Institution; 2018.
[17] Maillard J-Y, Messager S, Veillon R. Antimicrobial efficacy of biocides tested on skin using an ex-vivo test. J Hosp Infect 1998;40:313—23.
[18] BS EN 13697:2015+A1:2019. Chemical disinfectants and antiseptics, Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements without mechanical action (phase 2, step 2). London: British Standard Institute; 2019. https://doi.org/10.3403/30262906 [last accessed June 2025].
[19] ASTM E2967-15. Standard test method for assessing the ability of pre-wetted towelettes to remove and transfer bacterial contamination on hard, non-porous environmental surfaces using the wiperator. ASTM international; 2015. https://doi.org/10.1520/E2967-15 [last accessed June 2025].
[20] Lambert PA, Hammond SM. Potassium fluxes, first indications of membrane damage in micro-organisms. Biochem Biophys Res Commun 1973;54:796—9.
[21] Nie X, Liu W, Chen M, Liu M, Ao L. Flow cytometric assessment of the effects of chlorine, chloramine, and UV on bacteria by using nucleic acid stains and 5-cyano-2,3-ditolyltetrazolium chloride. Front Environ Sci Eng2016;10:12.
[22] Wesgate R, Robertson A, Barrell M, Teska P, Maillard JY. Impact of test protocols and material binding on the efficacy of antimicrobial wipes. J Hosp Infect 2019;103:E25—32.
[23] Wesgate R, Grascha P, Maillard J-Y. The use of a predictive protocol to measure the antimicrobial resistance risks associated with biocidal product usage. Am J Infect Control 2016;44:458—64.
[24] Sloan A, Kasloff SB, Cutts T. Mechanical wiping increases the efficacy of liquid disinfectants on SARS-CoV-2. Front Microbiol 2022;13:847313.
[25] Ramm L, Siani H, Wesgate R, Maillard J-Y. Pathogen transfer and high variability in pathogen removal by detergent wipes. Am J Infect Control 2015;43:724—8.
[26] Cutts TA, Robertson C, Theriault SS, Nims RW, Kasloff SB, Rubino JR, et al. Assessing the contributions of inactivation, removal, and transfer of Ebola virus and vesicular stomatitis virus by disinfectant pre-soaked wipes. Front Public Health 2020;8:183.
[27] Parveen N, Chowdhury S, Goel S. Environmental impacts of the widespread use of chlorine-based disinfectants during the COVID-19 pandemic. Environ Sci Pollut Res 2022;29:85742—60.
[28] Arnold WA, Blum A, Branyan J, Bruton TA, Carignan CC, Cortopassi G, et al. Quaternary ammonium compounds: a chemical class of emerging concern. Environ Sci Technol 2023;57:7645—65.
[29] Wong M-K, Chan T-C, Chan W-Y, Chan W-K, Vrijmoed LLP, O'Toole DK, et al. Dioxiranes generated in situ from pyruvates and Oxone as environmentally friendly oxidizing agents for disinfection. Environ Sci Technol 2006;40:625—30.
[30] Alvi S, Jayant V, Ali R. Applications of Oxone® in organic synthesis: an emerging green reagent of modern era. Chemistry Select 2022;7.
[31] Kim SM, Gwak EJ, Jeong SH, Lee SM, Sim WJ, Kim JS. Toxicity evaluation of cellulose nanofibers (Cnfs) for cosmetic industry application. J Toxicol Risk Assess 2019;5:29.
[32] Rizeq BR, Younes NN, Rasool K, Nasrallah GK. Synthesis, bioapplications, and toxicity evaluation of chitosan-based nanoparticles. Int J Mol Sci 2019;20:5776.
[33] De Oliveira KMG, Carvalho EHS, Pereira BS, Petersohn Jr E, Magalhães WLE, Moura RBP, et al. Testing the ecotoxicity of nanofibrillated kraft-bleached pulp for use in nanotechnology products. Cellulose 2025;32:519—34.
[34] 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.
[35] Tajeddin E, Rashidan M, Razaghi M, Javadi SSS, Sherafat SJ, Alebouyeh M, et al. The role of the intensive care unit environment and health-care workers in the transmission of bacteria associated with hospital acquired infections. J Infect Public Health 2016;9:13—23.
[36] Kundrapu S, Sunkesula V, Jury LA, Sitzlar BM, Donskey CJ. Daily disinfection of high-touch surfaces in isolation rooms to reduce contamination of healthcare workers' hands. Infect Control Hosp Epidemiol 2012;33:1039—42.