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Rosin as a natural alternative for the effective disinfection of ESKAPE pathogens and *Clostridioides difficile* spores

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Abstract

Aim Hospital-acquired infections (HAIs) caused by antimicrobial-resistant ESKAPE pathogens are a significant concern for the healthcare industry, with an estimated cost of up to $45 billion per year in the US alone. *Clostridioides difficile* is an additional opportunistic pathogen that also poses a serious threat to immunocompromised patients in hospitals. Infections caused by these pathogens lead to increased hospital stays and repeated readmission, resulting in a significant economic burden. Disinfectants and sporicidals are essential to reduce the risk of these pathogens in hospitals, but commercially available products can have a number of disadvantages including inefficacy, long contact times, short shelf lives, and operator health hazards. In this study, we evaluated the effectiveness of Rosin (a natural substance secreted by coniferous trees as a defence mechanism against wounds in tree bark) and its commercial derivative Rosetax-21 as disinfectants and sporicidal against the six ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) and spore preparations from *C. difficile*.

Methods and Results Both Rosin and Rosetax-21 were tested under simulated clean and dirty conditions [with Bovine Serum Albumin (BSA)] against the ESKAPE pathogens, and *C. difficile* spore preparations. In clean conditions, Rosin (5% weight/volume: w/v) demonstrated significant efficacy against five of the ESKAPE pathogens, with *A. baumannii* and *Enterococcus faecium* being the most susceptible, and *K. pneumoniae* the most resistant, showing only a 1-log reduction after a 5 min treatment. However, in dirty conditions, all pathogens including *K. pneumoniae* exhibited at least a 3-log reduction to Rosin within 5 min. Rosetax-21 (5% w/v) was found to be less effective than Rosin in clean conditions, a trend that was exacerbated in the presence of BSA. Additionally, both Rosin and Rosetax-21 at 2.5% (w/v) achieved complete eradication of *C. difficile* spores when combined with 0.5% glutaraldehyde, though their standalone sporicidal activity was limited.

Conclusions The findings from this study highlight the potential of Rosin and Rosetax-21 as both bactericidal and sporicidal disinfectants, with their efficacy varying based on the conditions and the pathogens tested. This presents an avenue for the development of novel healthcare disinfection strategies, especially against HAIs caused by antimicrobial-resistant ESKAPE pathogens and *C. difficile*.

Impact Statement

The escalating problem of healthcare-acquired infections (HAIs) due to antimicrobially resistant ESKAPE pathogens not only compromises patient safety but also imposes a hefty economic burden on the healthcare sector with the financial implications of HAIs reaching up to $45 billion annually in the USA. The opportunistic pathogen *Clostridioides difficile* further exacerbates this issue, especially for immunocompromised patients. This study describes the antimicrobial potential of Rosin and its derivative Rosetax-21, as a promising disinfectant and sporicidal against both the ESKAPE pathogens and *C. difficile* spores, respectively. The notable efficacy of Rosin, particularly in dirty conditions, and its sporicidal effectiveness when combined with glutaraldehyde, paves the way for potentially new disinfectant formulations for use in both clinical and non-clinical settings. The insights gleaned from this investigation underscore the potential of harnessing natural substances like Rosin in combating HAIs, thus contributing towards alleviating the associated economic burden and promoting a safer healthcare environment.

Keywords: antimicrobial; ESKAPE; *Clostridioides difficile*; spores; Rosin; plant extracts

Introduction

Hospital-acquired infections (HAIs) are a critical issue in healthcare, imposing an estimated annual cost of $45 billion in the USA alone (Barrasa-Villar et al. 2017, Boev and Kiss 2017). The situation is exacerbated by the escalating threat of antimicrobial resistance (AMR), recognized by the World Health Organization as a significant global health risk.

The primary strategy for mitigating HAIs is the rigorous decontamination of healthcare environments, with particular emphasis on high-frequency touch areas that serve as reservoirs for pathogenic microbes (Fawley et al. 2007, Dancer 2009, Hacek et al. 2010). However, the disinfectants currently in use often fall short in several key areas. Firstly, they are limited in their efficacy against bacterial spores, which are notoriously resilient to extreme conditions such as high temperatures and chemical agents. Additionally, these commercial disinfectants often require extended contact times, have limited shelf lives, and can be both environmentally

detrimental, and hazardous to human health (Uwamahoro et al. 2018).

Considering these challenges, there is a need for alternative disinfectants and sporicidals that are both effective and safe. Rosin, a natural substance secreted from coniferous trees as a defence mechanism against wounds in tree bark, offers a promising direction for exploration. Rosin consists of a complex mixture of sodium salts and acids including, abietic acid, dehydroabietic acid, pimaric acid, and palustric acid and has previously been shown to exhibit antibacterial properties, especially against Gram-positive bacteria (Söderberg et al. 1990, Savluchinske-Feio et al. 2006).

This study aims to further extend the current understanding of Rosin's antimicrobial capabilities by investigating its efficacy against the ESKAPE pathogens (*Enterococcus pneumonaiee*, *Staphylococcus aureus*, *Klebsiella pneumonaiee*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), as well as spores prepared from cells of *Clostridioides difficile*. Individually and in combination, these pathogens are a frequent cause of HAIs and have shown a concerning tendency to develop multidrug resistance, thereby extending hospital stays and posing a significant threat to immunocompromised and elderly patients (Pendleton et al. 2013). For example, methicillin-resistant *S. aureus* (MRSA) contributed to 28 424 deaths in England and Wales from 1993 to 2011 (Office for National Statistics 2014), while *C. difficile* infection is a leading cause of gastroenteritis-associated deaths and currently the most common cause of HAI in many developed countries (Miller et al. 2011, Hall et al. 2012). This study seeks to advance the field of hospital disinfection by providing a comprehensive evaluation of Rosin and Rosetax-21, focusing on their efficacy against ESKAPE pathogens and *C. difficile* spores, combating the dual challenges of HAIs and AMR.

Experimental procedures

Tall oil derivatives

In this study, samples of Rosin and Rosetax-21 were provided by Forchem Ltd. (Rauma, Finland) as a dried salt—produced from crude tall oil. The supplied Rosin consisted of <10% sodium salts of tall oil fatty acids and >90% sodium salts of rosin acids. The Rosetax-21 is a derivative consisting of potassium salt of tall oil fatty acids, including 21% rosin acids, 24% ethanol, and 11% water. The substances originate from the coniferous trees *Pinus sylvestris* L. and *Picea abies* L., with a composition primarily featuring abietic acid, dehydroabietic acid, pimaric acid, and palustris acid.

The Rosin and Rosetax-21 products were reconstituted as a 10% (w/v) solution in deionized water and the pH adjusted to pH 7–7.5. All characterization of samples was conducted and validated by Forchem Ltd. (Rauma, Finland). These methods allow for a precise definition of 'rosin' as used in our experiments, acknowledging the inherent variability in natural products.

Bacterial strains and growth conditions

Enterococcus faecium DSM 25390, MRSA ATCC 33592, *K. pneumonaie* NCTC 204, *A. baumannii* NCTC 13304, *P. aeruginosa* PA01, and *Enterobacter cloacae* NCTC 5920 (collectively referred to as the ESKAPE pathogens), were incubated at 37°C on Mueller–Hinton agar (MHA). *Clostridioides*

difficile strains TL178 (Clade 1, Ribotype 002), and CD305 (Clade 3, Ribotype 023), known to produce toxins A and B (Yang et al. 2014), were obtained from the Kelvin Laboratories at the Royal Victoria Hospital, Belfast. These strains have been previously characterized for their resistance to non-thermal plasma exposure, providing insights into their evolutionary clade-associated susceptibility patterns (Connor et al. 2017). The cultures were incubated at 37°C in a Whitley A35 anaerobic workstation on pre-reduced fastidious anaerobe agar (FAABL: Oxoid, UK).

Clostridioides difficile spore preparation

Clostridioides difficile spore suspensions were prepared according to the method described by Wheeldon et al. (2008). Individual FAABL agar plates were inoculated with cells of TL178 and CD305 and incubated anaerobically (N_2, CO_2, H_2) at 37°C for 72 h in the anaerobic chamber. Plates were subsequently incubated under aerobic conditions for 24 h at 37°C following which colonies were harvested into 5 ml sterile phosphate-buffered saline (PBS) and shocked with absolute ethanol 50% for 1 h to kill vegetative cells (Wheeldon et al. 2008). The alcohol-shocked suspensions were centrifuged at $3000 \times g$ for 4 min and washed once in 5 ml sterile PBS with the resultant pellet was resuspended in 70% ethanol. Spore concentration was determined as $\sim 1.3 \times 10^7$ spores/ml, by viable spore counts.

Evaluating the antimicrobial efficacy of the Rosin formulations against ESKAPE pathogens

The effect of Rosin and Rosetax-21 on the ESKAPE pathogens was assayed according to British Standard BS EN 13704:2018. Colonies of each ESKAPE pathogen were suspended in quarter-strength ringers' solution (QSRS), to an OD_{550} (optical density, 550 nm) of 0.3 and then further diluted 1 in 50 in QSRS, resulting in 10^6 CFU/ml [0.6% Bovine Serum Albumin (BSA) was added to the QSRS to simulate dirty conditions]. Next, 100 µl of cell suspension was mixed with either Rosin or Rosetax-21 to a final concentration of either 2.5% or 5% w/v and incubated for various time points (30 s, 1 min, 2 min, 3 min, 4 min, and 5 min). At each time point, 20 µl of the mixture was withdrawn and diluted to 10^{-4}, and the diluted samples were spotted onto MHA and incubated for 18 h at 37°C to determine the CFU/ml.

To account for any changes in bacterial growth during the experiment, a time zero control was prepared by mixing 100 µl of bacterial suspension with 100 µl of QSRS instead of Rosin and Rosetax-21. The time zero control was also serially dilute to 10^{-4} as described above and spotted onto nutrient agar to determine the CFU/ml at the start of the experiment. A neutralizer control (Dey Engley neutralizing broth) was also included to ensure that the neutralizer buffer did not interfere with the activity of the Rosin preparations. To perform the neutralizer control, 100 µl of the test substance was added to wells in a 96-well plate, and then, 100 µl of neutralizer buffer was added to the same well and mixed well. Next, 100 µl of the mixture from the first well was transferred to a second well containing 100 µl of a cell suspension with a concentration of $\sim 10^6$ CFU/ml. The mixture was incubated for 5 min, after which 20 µl was withdrawn and serially diluted to 10^{-4}, spotted onto nutrient agar plates and incubated for 18 h at 37°C to determine the CFU/ml.

Table 1. Chemical disruptor solutions and their mechanisms.

Chemical disruptor	Concentration (w/v)	Contact time	Mechanism
Glutaraldehyde	0.5%	10 min	Alkylation of nucleophilic groups in proteins and DNA
Sodium dichloroisocyanurate (NaDCC)	0.05%	10 min	Chlorination of cellular components
Hydrogen peroxide (H_2O_2)	4%	10 min	Oxidative damage to cellular components
N-methylpyrrolidine	5%	10 min	Disruption of cell membrane integrity
2-pyrrolidone	5%	10 min	Solubilization of membrane lipids
Dimethylacetamide	5%	10 min	Protein denaturation
Glycerol formal	5%	10 min	Dehydration and denaturation of proteins
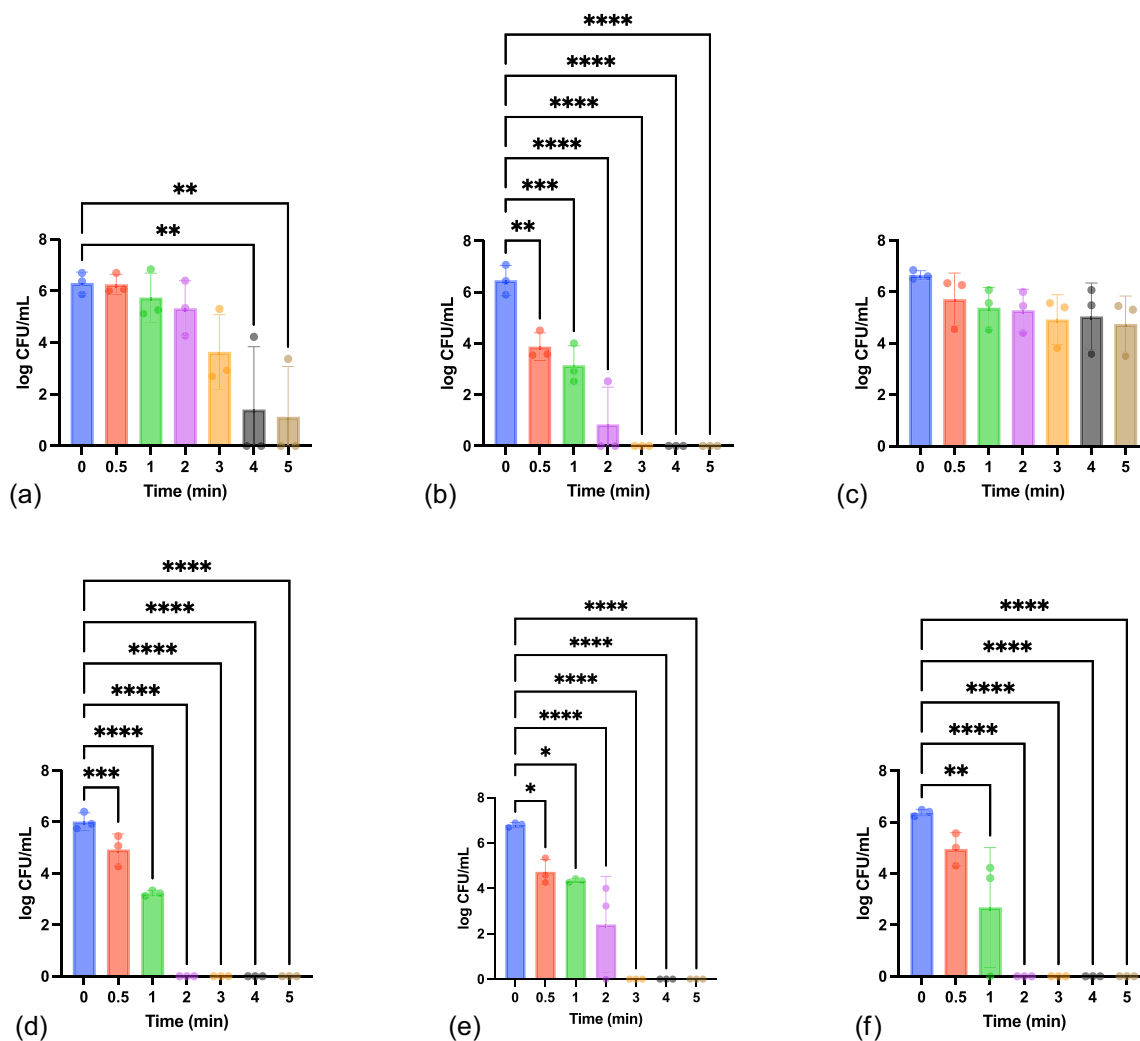
Figure 1. Effect of Rosin (5% w/v) after 0-, 0.5-, 1-, 2-, 3-, 4-, and 5-min exposure against *Enterobacter cloacae* (a), MRSA (b), *K. pneumonaie* (c), *A. baumannii* (d), *P. aeruginosa* (e), and *Enterococcus faecium* (f). Results are the means of three biological replicates, with error bars representing standard error of the mean. ($n = 3$).

Assessment of sporicidal activity

To determine the effect of Rosin and Rosetax-21 on *C. difficile* spores, 100 µl of spores (ca. 1300 CFU) were suspended in 100 µl of either Rosin or Rosetax-21 at a final concentration of 2.5%, and incubated for 10 min. Rosin and Rosetax-21 suspensions were also augmented with a range of chemical disruptors to aid sporicidal efficacy as described in Table 1.

Disruptors were tested in the absence of Rosin and Rosetax-21 as controls. Suspensions were incubated for 0 and 8 min after which time treated spores were serially diluted to 10^{-4} in Dey Engley neutralizing broth. Diluted samples were subsequently cultured on Brazier's medium (Fannin, Ireland) anaerobically at 37°C for 48 h with the number of colonies at each time point recorded to determine the CFU/ml.
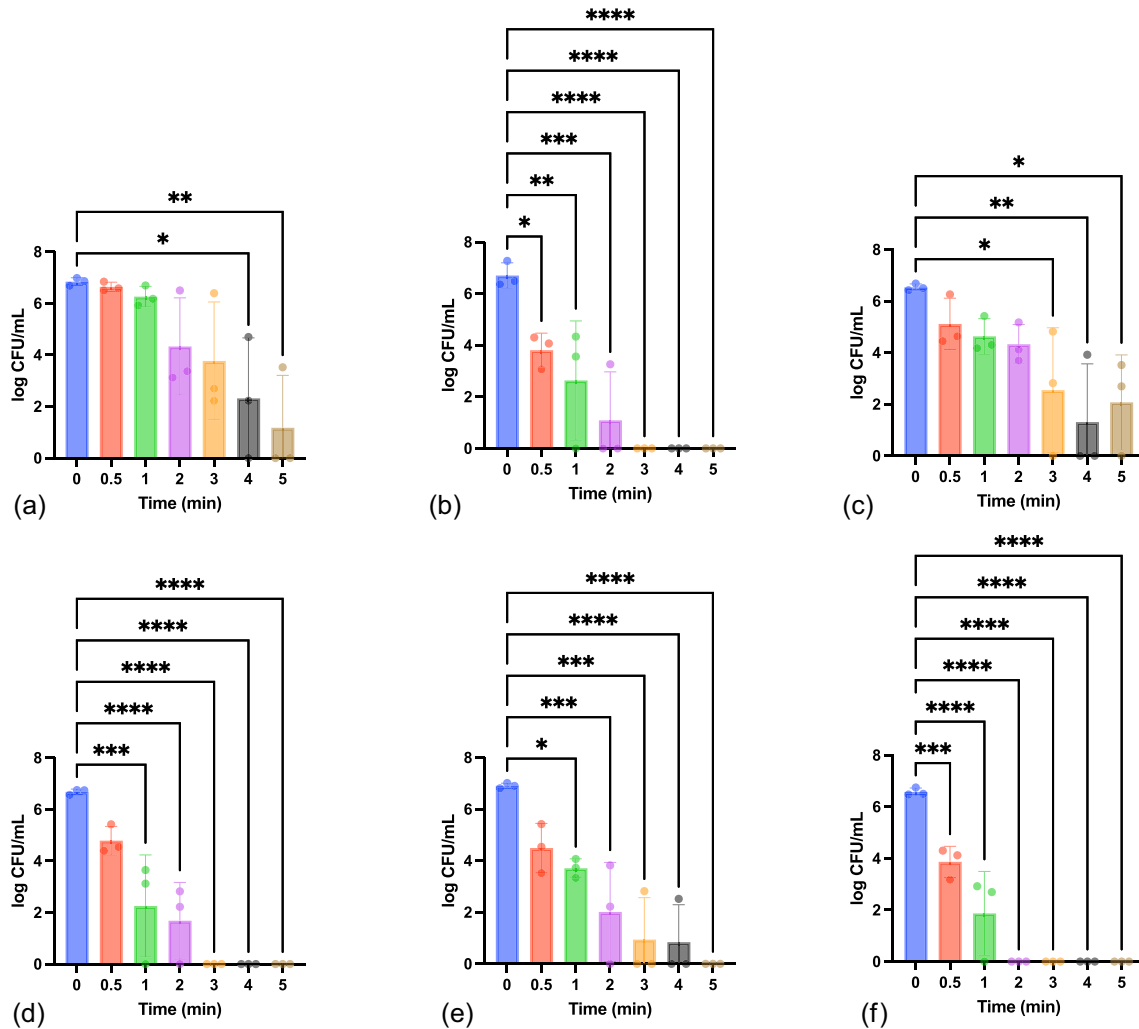
Figure 2. Effect of Rosin (5% w/v) in the presence of 0.6% BSA after 0-, 0.5-, 1-, 2-, 3-, 4-, and 5-min exposure against *Enterobacter cloacae* (a), MRSA (b), *K. pneumonaie* (c), *A. baumannii* (d), *P. aeruginosa* (e), and *Enterococcus faecium* (f). Results are the means of three biological replicates, with error bars representing standard error of the mean. ($n = 3$).

Statistical analysis

The results for the average means and standard deviation from biological triplicates were compared using a two-way ANOVA with Dunnett's test against the untreated control were used. *P*-values of * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), and **** ($P \leq 0.0001$) indicate statistical significance. GraphPad Prism 10.1.1 was used for all analysis.

Results

Efficacy of Rosin against the ESKAPE pathogens in simulated clean conditions

Rosin (5% w/v) was tested against the six ESKAPE pathogens, five of which (*Enterobacter cloacae*, MRSA, *A. baumannii*, and *P. aeruginosa*) showed at least a 3-log reduction following 5-min exposure (Fig. 1). The greatest efficacy was observed against *A. baumannii* and *Enterococcus faecium*, with cessation of growth observed after 1-min exposure. No further bacterial growth was seen after 2-min exposure for both MRSA, and *P. aeruginosa*. *Enterobacter cloacae* was not killed within 5 min; however, a 3-log reduction in colony numbers occurred

between 0 and 5 min. *Klebsiella pneumoniae* was most resistant to treatment by Rosin, with an observed 1-log reduction in colony numbers after 5 min treatment.

Efficacy of Rosin against the ESKAPE pathogens in simulated dirty conditions

For any disinfection study, it is important to determine the effect of inferring substances on product efficacy by introducing an organic contaminant such as BSA into the disinfection assay. To this end, the antimicrobial activity of the resin acids was re-assessed in the presence of BSA (6 g/l): the British standard for organic contamination is 3 g/l BSA. Rosin (5% w/v) in the presence of BSA exhibited at least a 3-log reduction with all six ESKAPE pathogens following 5-min exposure (Fig. 2). The greatest efficacy was observed against *Enterococcus faecium*, with cessation of growth noted after 1-min exposure. After 2 min of exposure, there was complete eradication of MRSA, *A. baumannii*, and *P. aeruginosa*. *Enterobacter cloacae* and *K. pneumonaie* were not killed within 5 min; however, a 3-log reduction in CFU/ml was observed. We observed a unique response of *K. pneumonaie* to Rosin in the presence
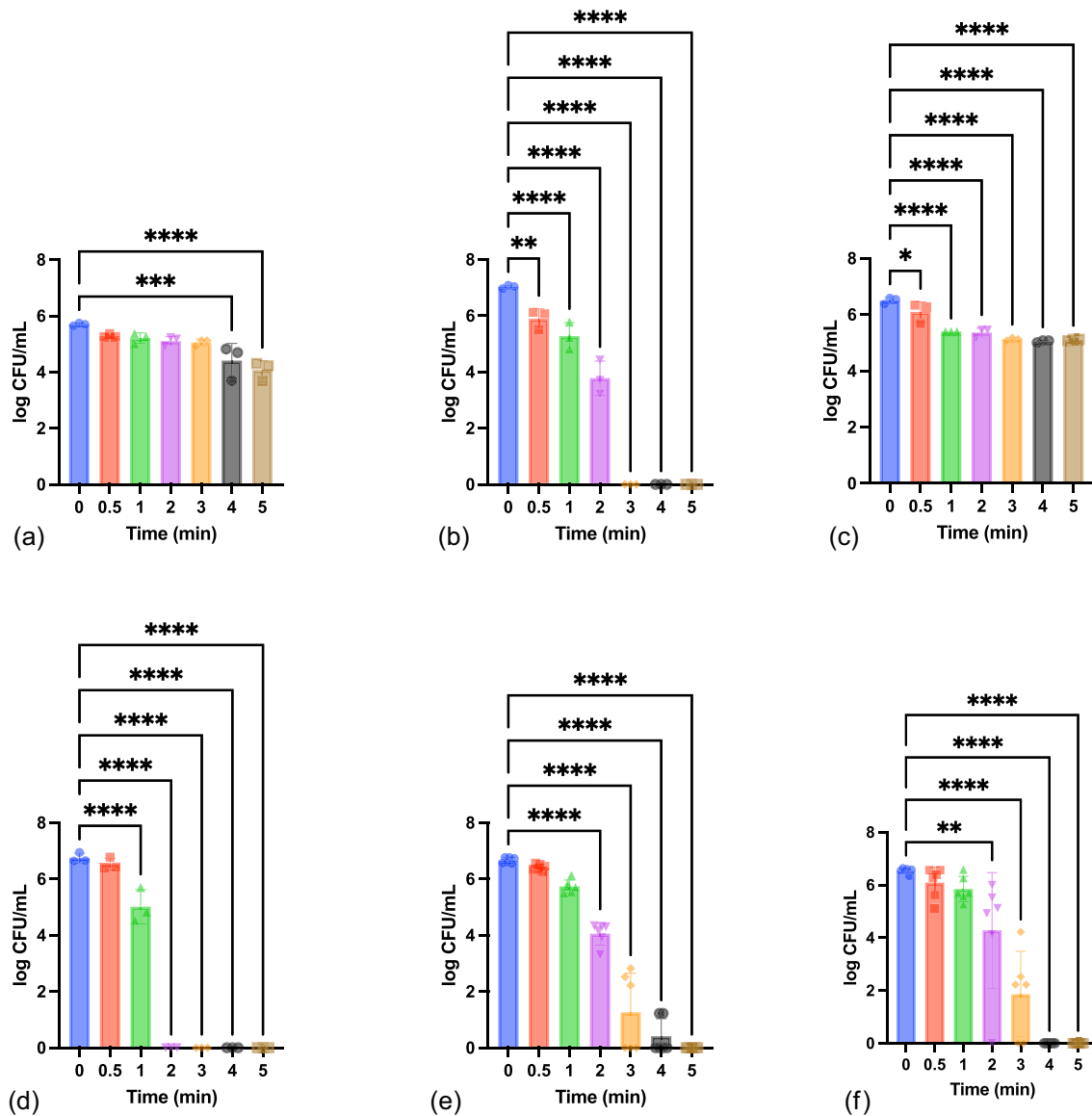
Figure 3. Effect of Rosetax-21 (5% w/v) after 0-, 0.5-, 1-, 2-, 3-, 4-, and 5-min exposure against *Enterobacter cloacae* (a), MRSA (b), *K. pneumonaie* (c), *A. baumannii* (d), *P. aeruginosa* (e), and *Enterococcus faecium* (f). Results are the means of three biological replicates, with error bars representing standard error of the mean. ($n = 3$).

of organic matter. While most ESKAPE pathogens typically exhibit reduced susceptibility to disinfectants in the presence of organic contaminants, *K. pneumonaie* showed an enhanced response to Rosin in these conditions. This suggests that there might be specific interactions or factors inherent to *K. pneumonaie* that make it more susceptible to Rosin when organic matter is present.

Efficacy of Rosetax-21 against the ESKAPE pathogens in simulated clean conditions

Next, we screened the Rosetax-21 (5% w/v) against the six ESKAPE pathogens, five of which (*Enterobacter cloacae*, MRSA, *A. baumannii*, *P. aeruginosa*, and *Enterococcus faecium*) showed at least a 2-log reduction following 5-min exposures (Fig. 3). The Rosetax-21 (5% w/v), had a reduced bactericidal activity against all the ESKAPE pathogens when compared to the Rosin (5% w/v) formulation. For example,

Enterobacter cloacae was not as susceptible achieving only a 2-log reduction after 5 min treatment compared to > 6-log reduction observed with Rosin (5% w/v). *Klebsiella pneumoniae* remained as the most tolerant pathogen with only a 1-log reduction after 5 min treatment.

A comparison of the Rosteax-21 (5% w/v) and Rosteax-21 (2.5% w/v) formulations illustrates that the lower strength formulation exhibited the same spectrum of activity against the ESKAPE pathogens but at a reduced antibacterial activity (Fig. 4). For example, *Enterobacter cloacae*, MRSA, and *P. aeruginosa* had > 7-log reduction within 3 min treatment time with Rosteax-21 (5% w/v), while with Rosteax-21 (2.5% w/v), the treatment time required for similar efficacy increased to 4 min for *Enterobacter cloacae*, MRSA, and up to 5 min for *P. aeruginosa*. Unsurprisingly, *K. pneumonaie* and *Enterococcus faecium* appeared to be slightly less effective at the 2.5% w/v formulation.
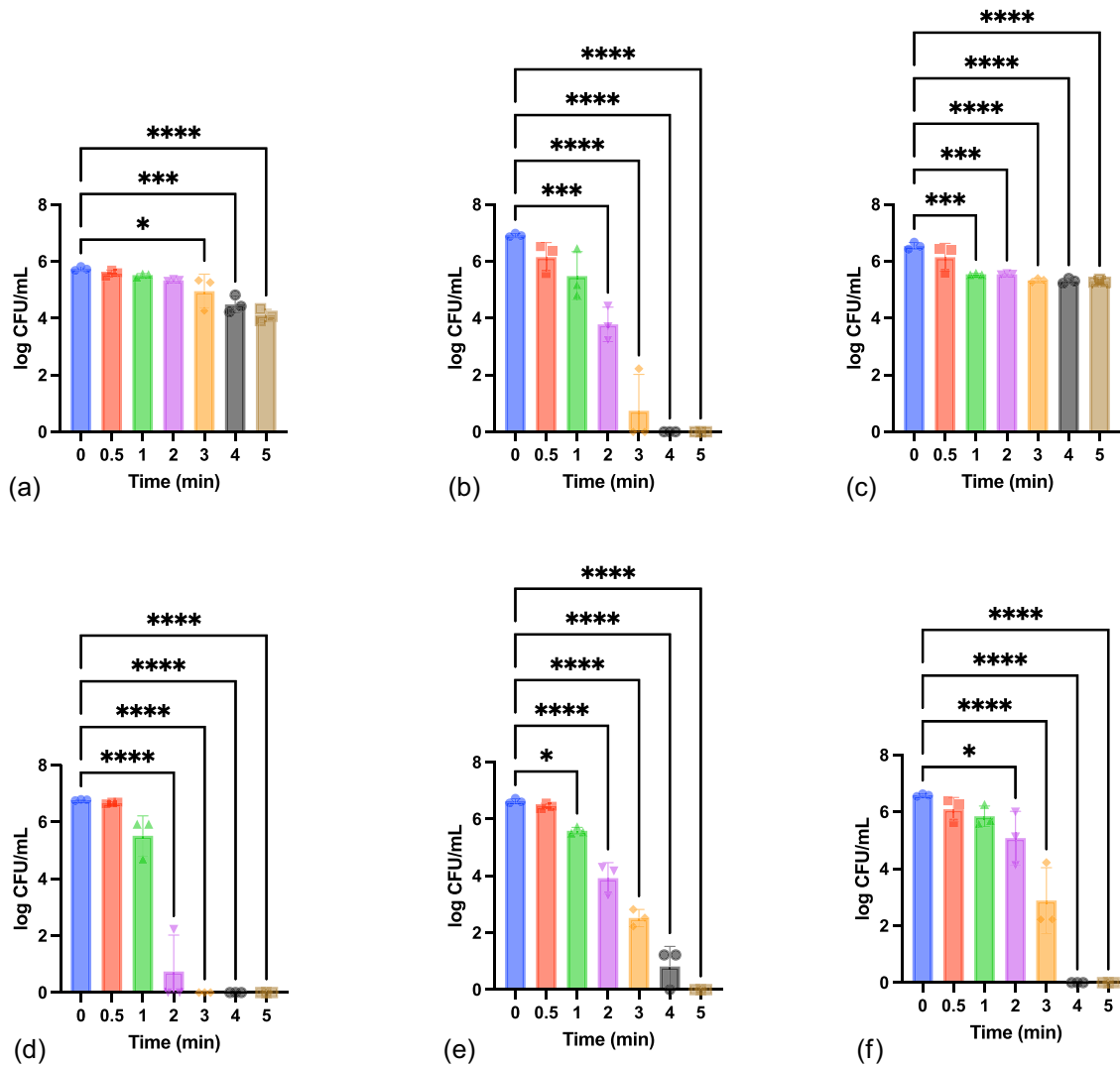
Figure 4. Effect of Rosetax-21 (2.5% w/v) after 0-, 0.5-, 1-, 2-, 3-, 4-, and 5-min exposure against *Enterobacter cloacae* (a), MRSA (b), *K. pneumonaie* (c), *A. baumannii* (d), *P. aeruginosa* (e), and *Enterococcus faecium* (f). Results are the means of three biological replicates, with error bars representing standard error of the mean. ($n = 3$).

Efficacy of Rosetax-21 against the ESKAPE pathogens in simulated dirty conditions

We then assessed the impact of dirty conditions on the efficacy of the Rosetax-21. Compared to the clean conditions, there was a reduction in antibacterial effect of Rosetax-21 at both 5% w/v (Fig. 5) and 2.5% w/v (Fig. 6). For example, total eradication of *Enterobacter cloacae* and MRSA was only possible with Rosetax-21 (5% w/v) with a treatment time of 5 min, illustrating the impact of BSA on the bactericidal efficacy of this formulation. Rosetax-21 appeared to retain some activity against *A. baumannii*, which required 3 min for 5% w/v and 4 min for 2.5% w/v formulations to attain complete eradication—both 1 min longer compared to clean conditions. Dirty conditions had no impact on the susceptibility of *K. pneumoniae* and *Enterococcus faecium*, both pathogens were resistant to treatment at both concentrations.

Efficacy of Rosetax-21 (2.5% w/v) and Rosin (2.5% w/v) against spores of *C. difficile*

Both Rosetax-21 (2.5% w/v) and Rosin (2.5% w/v), when used in combination with a sublethal concentration of glutaraldehyde (0.5%), completely inactivated spores from two different clades of *C. difficile* (Fig. 7). In contrast, Rosetax-21 and Rosin, when tested in the absence of glutaraldehyde (0.5%) did not significantly reduce spore counts (Fig. 7). Additionally, glutaraldehyde (0.5%) without the inclusion of Rosetax-21 or Rosin was not effective at reducing spore numbers (Fig. 7). The use of Rosetax-21 or Rosin in combination with other chemical disrupters including sodium dichloroisocyanurate (0.05%), hydrogen peroxide (4%), *N*-methylpyrrolidine (5%), 2-pyrrolidine (5%), dimethylacetamide (5%), and glycerol formal (5%) did not result in any reduction in spore counts (results not shown).
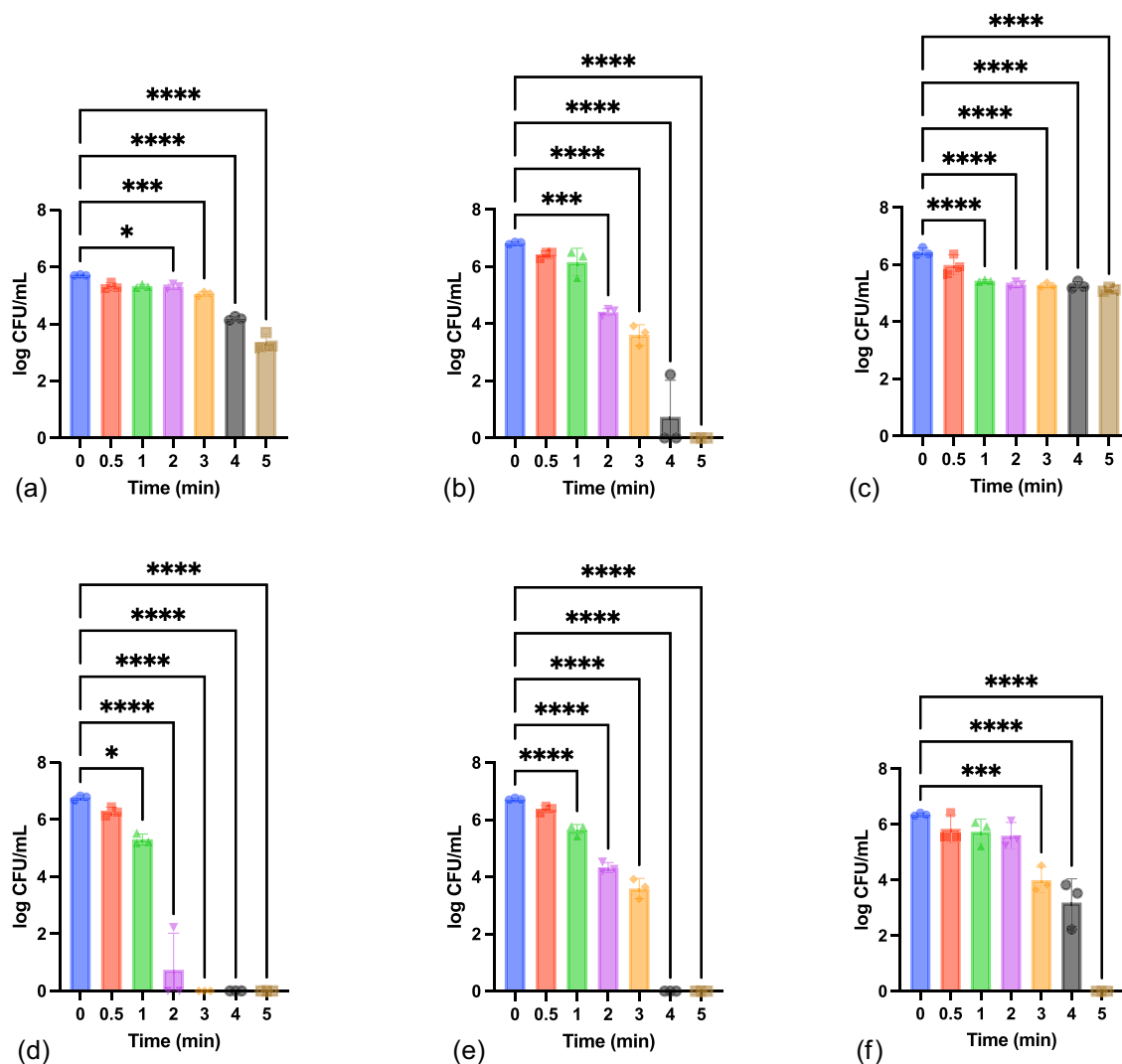
Figure 5. Effect of Rosetax-21 (5% w/v) in the presence of 0.6% BSA after 0-, 0.5-, 1-, 2-, 3-, 4- and 5-min exposure against *Enterobacter cloacae* (a), MRSA (b), *K. pneumonaie* (c), *A. baumannii* (d), *P. aeruginosa* (e), and *Enterococcus faecium* (f). Results are the means of three biological replicates, with error bars representing standard error of the mean. ($n = 3$).

Discussion

Many commercially available disinfectant and sporicidal products, registered for use in hospital settings, come with significant disadvantages. The primary concern is the high levels of chlorine used within formulations, with concentrations of at least 1000 ppm free chlorine necessary for effective pathogen destruction and spore killing. Given that the UK Health and Safety Executive recommends an exposure limit of 0.5 ppm chlorine, the health implications for operators using such disinfectants are evident (Coates 1996, Health 2008, Wheeldon et al. 2008, Health and Safety Executive 2011). Furthermore, many of these products have limited shelf lives, necessitate dedicated disposal routes due to their environmental toxicity, and can be costly. Many products within the marketplace have no to low efficacy with many requiring prolonged exposure times ranging from 2 to 25 min (see Supplementary Table S1). Speight et al. (2011) highlighted that only 50% of the commercial sporicidal products tested were effective against *C. difficile* after 60 min, and only eight

products showed efficacy after 1 min. There is an urgent need for safer and more effective disinfectants for the treatment of pathogen and spores (Speight et al. 2011).

Rosin, a natural oil derived from pine trees, offers advantages over traditional chemical disinfectants. While there is previous evidence of skin sensitization and respiratory risks associated with rosin constituents at concentrations of > 20% (Forschungsgemeinschaft. 2013), our formulations of Rosin and Rosetax-21 are used at lower concentrations (5% and 2.5%), indicating a lower potential for sensitization with certain derivatives, which may mitigate these risks(Illing et al. 2009). Moreover, we demonstrate the broad antimicrobial spectrum of both Rosin and Rosetax-21, achieving full eradication of *A. baumannii* and *Enterococcus faecium* at 3 and 2 min, respectively. This is within the contact times required by more toxic chloride-based products (Supplementary Table S1). These contact times reduce user handling time and thereby improve user safety, and their nonchloride components reduce environmental impact. Additionally, the
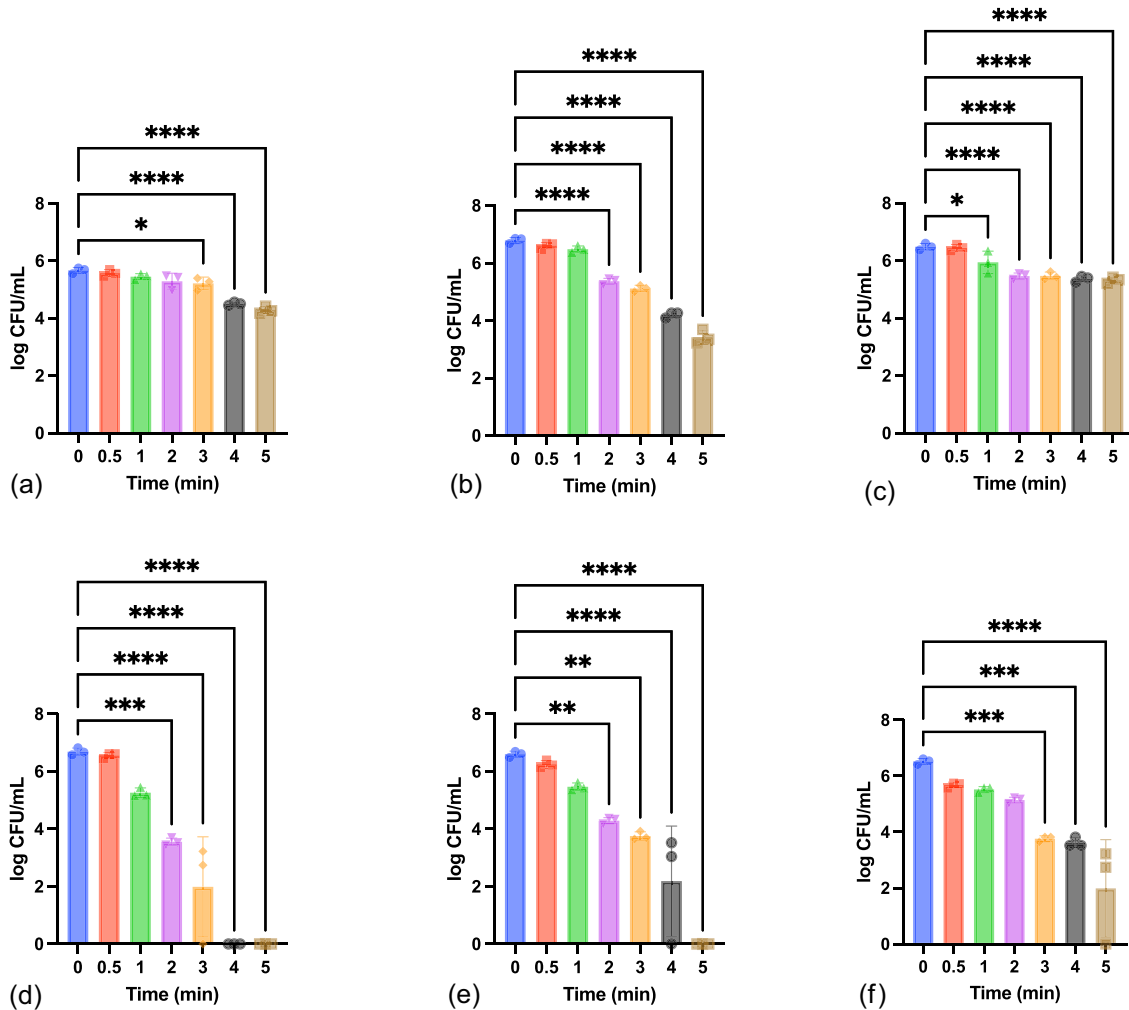
Figure 6. Effect of Rosetax-21 (2.5% w/v) in the presence of 0.6% BSA after 0-, 0.5-, 1-, 2-, 3-, 4-, and 5-min exposure against *Enterobacter cloacae* (a), MRSA (b), *K. pneumoniae* (c), *A. baumannii* (d), *P. aeruginosa* (e), and *Enterococcus faecium* (f). Results are the means of three biological replicates, with error bars representing standard error of the mean. ($n = 3$).

rosin-based products retained bioactivity even in the presence of organic contaminants, indicating their robust performance under varied environmental conditions.

Our findings align with the previous studies, where rosin displayed antimicrobial against Gram-positive (*S. aureus*, and MRSA) and Gram-negative (*P. aeruginosa*) bacteria (Sipponen and Laitinen 2011). Furthermore, Santovito et al. demonstrated that rosin acids loaded into nanoparticles displayed enhanced antimicrobial properties especially against *Clostridium perfringens*, *Listeria monocytogenes*, and antibiotic-resistant *S. aureus*. However, they were ineffective against Gram-negative pathogens like *Campylobacter jejuni*, *Camp. coli*, *Escherichia coli*, and *Salmonella enterica* (Santovito et al. 2018). The enhanced efficacy of rosin loaded into nanoparticles suggests potential avenues for further optimizing rosin-based formulations against the ESKAPE pathogens. Recent work by Leite et al. showcased rosin's versatility by incorporating rosin-grafted cellulose nanocrystals into gelatin films for antimicrobial food packaging. Their findings highlighted rosin's potential in nonclinical applications (Leite et al. 2020).

Beyond antimicrobial activity, we observed a synergetic sporicidal effect when Rosin and Rosetax-21 were used in combination with a 0.5% concentration of glutaraldehyde against two distinct clades of *C. difficile* spores. This observation is particularly notable given that glutaraldehyde is typically sporicidal at concentrations >2.4% (Sykes and Weese 2014). This synergy suggests that lower concentrations of glutaraldehyde may be used effectively in conjunction with rosin-based compounds, enhancing the safety profile for operators. Interestingly, such an enhancement was not observed when rosin-based compounds were used with other chemical disrupters. Further research is needed to elucidate the mechanisms underlying the synergistic effects observed with the Rosin–glutaraldehyde and Rosetax-21–glutaraldehyde mixtures.

The natural origin of Rosin and rosin products with demonstrated antimicrobial properties across various pathogens, including the ESKAPE pathogens, *C. difficile* spores, and enveloped viruses like influenza A and SARS-CoV-2, as demonstrated in Bell et al. (2021), alongside reported anti-inflammatory activity, present Rosin as a natural
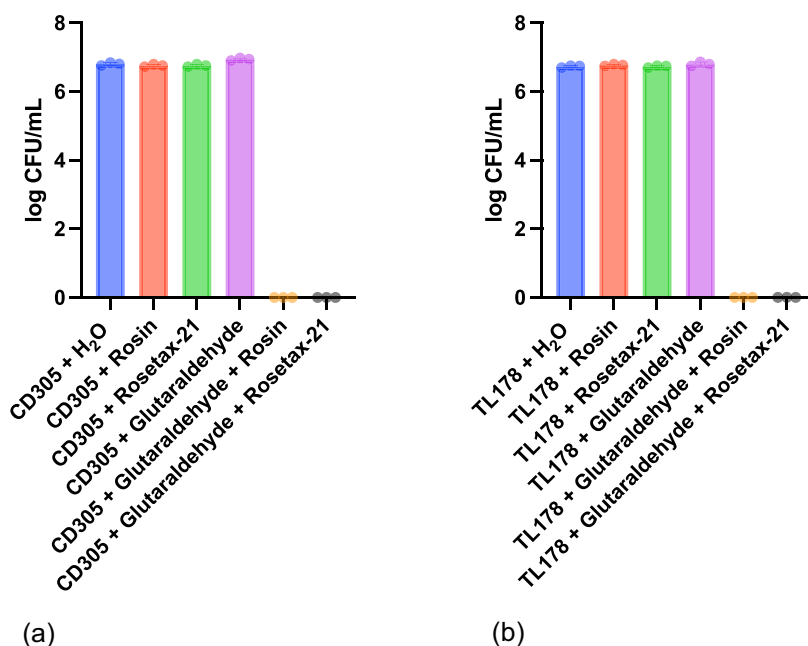
Figure 7. Effect of Rosetax-21 (2.5% w/v) and Rosin (2.5% w/v) with 10-min exposure with and without the presence of glutaraldehyde (0.5%) against *C. difficile* (a), CD305 (b), and TL178. H$_2$O treatment served as an untreated control. Results are the means of three biological replicates. ($n = 3$).

alternative for potential future disinfectant and sporicidal formulations.

Supplementary data

Supplementary data is available at *JAMBIO Journal* online.

Conflict of interest: All authors have no conflicts of interest to declare.

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Author contributions

Stephen Bell (Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft), Thomas P. Thompson (Data curation, Formal analysis, Methodology, Writing – original draft), Nikki Marks (Supervision), Derek Fairley (Project administration, Supervision), Hannele Kettunen (Conceptualization, Funding acquisition, Writing – review & editing), Juhani Vuorenmaa (Conceptualization, Funding acquisition, Writing – review & editing), Juha Orte (Conceptualization, Funding acquisition, Writing – review & editing), Brendan F. Gilmore (Supervision), and John W. McGrath (Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – review & editing)

Data availability

All data generated or analysed during this study are included. No animals, patient samples, or human tissue were used in this research. No patient data were collected or analysed. All research involved *in vitro* laboratory experiments.

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