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Research Article

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Determining the efficacy of disinfectants at nucleic acid degradation

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Abstract

Aims: Nucleic acids, particularly antibiotic resistance genes, are commonly found on surfaces within healthcare environments, with levels not reducing following cleaning. Within the UK, there are no regulations for testing disinfectants against nucleic acids.

Methods and results: A series of commonplace *in vitro* methods were used to determine disinfectant-induced physical and functional damage to various nucleic acids; RNA (10 μg), genomic DNA (2 μg), and plasmids (1 μg). Using these methods, the optimal residence time (10 minutes) and working concentration (10%) were determined for a new disinfectant. Furthermore, comparison of disinfectants with different active ingredients including lactic acid (LA), sodium hydroxide (NaOH), chloroxylenol (PCMX), and quaternary ammonium compounds (QACs), were compared to controls. All disinfectants showed greater degradation by gel electrophoresis of genomic DNA and RNA than of purified plasmids. Functional analysis using quantitative polymerase chain reaction (qPCR) and polymerase chain reaction (PCR) demonstrated that no disinfectant tested (apart from control) could damage DNA to the level where PCR amplification was not possible, and only the NaOH reagent could achieve this for RNA.

Conclusions: The set of methods described herein provides a platform for future standardization and potential regulation regarding monitoring cleaning solutions for their activity against nucleic acids.

Impact Statement

By analyzing the effect of the product across several parameters, manufacturers can produce clear claims to offer users a better overview for how products compare.

Keywords: disinfection, degradation, antimicrobial resistance, genes, nucleic acid

Introduction

Disinfectants, biocidal chemicals that are used to control contamination by micro-organisms, are used as cleaning products in a wide range of environments from offices to operating theatres. Currently, within the UK and EU, there are comprehensive regulations regarding the process of bringing a disinfectant to market. This involves strict testing for antimicrobial activity, following the European Committee for Normalization (CEN) technical committee 216 work programme with standardized methods to validate the disinfectants efficacy (Chemical disinfectants and antiseptics-Application of European Standards for chemical disinfectants and antiseptics, 2023). To date, however, there are no regulations or guidance available for the testing of disinfectants against nucleic acids. Many current cleaning methods are not sufficient to clear nucleic acids from contaminated surfaces. In 2015, one study showed that although cleaned surfaces within a hospital environment were less likely to have cultivable target bacterial species than pre-cleaned surfaces, and yet they still contained similar levels of bacterial DNA (Lesho et al. 2015). Furthermore, the level of DNA that can be isolated from inanimate surfaces can be reasonably high with 2.82 ng μL^{-1} recovered from steering wheels (Comte et al. 2019).

The persistence of nucleic acids on surfaces is important due to the threat of antibiotic resistance genes (ARGs), pathogenicity islands, toxin-encoding genes, or viral genomes. The resistance mechanisms to antibiotics are encoded within ARGs, which facilitate the various mechanisms required for bacterial survival in the presence of antibiotics. Following lysis of the infectious agents these oligonucleotides can be distributed intact or within membrane vesicles within the vicinity of the lysed cells (Muschiol et al. 2015, Turnbull et al. 2016). Bacteria can take up exogeneous DNA by three main methods: conjugation, transduction, and transformation (Soucy et al. 2015). The latter of these methods involves the uptake of DNA in a sequence non-specific manner and has been observed in both bacteria and archaea (Fuchsman et al. 2017). It can result in bacteria acquiring genes encoding toxins, genes involved in antibiotic resistance, or even whole pathogenicity islands (Sun 2018). Some naturally competent bacteria including *Bacillus subtilis* and *Acinetobacter* will take up exogenous DNA from any source with the same efficiencies, whereas other bacterial species such as *Haemophilus influenzae* prefer-

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entially take up DNA from their own or related species (Kelly et al. 2009).

Infectious nucleic acids have been reported throughout literature, in particular relating to that of viral origin (Gerber 1962, Watts et al. 1983, Graham 1984). Viral nucleic acids have been found to be infectious in tissue and animal models and yet do not hold antigenic properties (Herriott 1961). Furthermore, the World Health Organisation (WHO) recommends 'acceptable limits of residual DNA' with regards to the potential of residual DNA encoding infectious agents (Yang 2013). In part this is linked to the persistence of HIV-1 DNA in patients on antiretroviral therapy, after the viral load has become undetectable, with residual DNA deemed a potential reservoir and therefore marker for the disease (Williams et al. 2014).

Apart from reagents to prevent polymerase chain reaction (PCR) contamination within laboratories, there has been a paucity of research focussed on the ability of disinfectants to denature or destabilize oligonucleotides on surfaces. Current literature investigating nucleic acid degradation by these agents has largely drawn on their impact on downstream experiments such as PCR or quantitative PCR (Esser et al. 2006, Fischer et al. 2016, Stoufer et al. 2023, Zhang et al. 2023). Tests for the activity of reagents against nucleic acids can be in solution or surface tests, with the latter being more applicable for the method of use for most commonplace disinfectants.

Here we investigate the use of a standard set of tests, composed of *in vitro* analysis methods (gel electrophoresis, PCR, qPCR, and chemical transformation) to best elucidate nucleic acid-degrading properties of a disinfectant. We demonstrate this by determining the optimal parameters for an exemplar disinfectant and by showing how these methods can be used to compare the ability of disinfectants to degrade nucleic acids.

Materials and methods

Throughout this study, all the methods were tested against the following: a product designed to remove DNA contamination for PCR analysis in laboratories [active ingredient: sodium hydroxide (NaOH)]; a disinfectant claiming to degrade nucleic acids [active ingredient: quaternary ammonium compounds (QACs)]; and a commonly used household disinfectant [active ingredient: chloroxylenol (PCMX)]. Products were compared to known controls of nuclease-free water as a negative control and 10% sodium hypochlorite (NaOCl), previously shown to strongly degrade nucleic acids (Prince and Andrus 1992, Fischer et al. 2016), as a positive control.

Nucleic acid purification

Bacterial genomic DNA (gDNA) was extracted from *Escherichia coli* DH5α, grown in Luria- Bertani (LB) broth at 37°C, using GenElute™ bacterial gDNA kit (Sigma–Aldrich Co. LLC, UK) as per the manufacturer's instructions. The plasmid pUC19 was transformed into competent *E. coli* DH5α, colony selected and grown overnight in LB broth at 37°C, before plasmid extraction and purification using a GenElute™ Plasmid Maxiprep kit (Sigma–Aldrich Co. LLC, UK) as per the manufacturer's instructions. Total RNA was extracted from bacterial cells (*E. coli* DH5α) and mammalian cells (lymphoblast TK6) using a Cytiva RNAspin Mini isolation kit (Fisher Scientific, UK) as per manufacturer's instructions. All purified nucleic acids were quantified using absorbance at 260 nm readings, as measured using a NanoDrop ND-1000 spectrophotometer (Labtech).

Surface tests

Unless stated otherwise for DNA stability tests, 1 μg (from ≤ 10 μL in volume) of the purified plasmid pUC19 or gDNA was added to microcentrifuge tubes and left to dry at room temperature in a laminar flow hood overnight, with all conditions tested in triplicate. Alternatively, 10 μg (from ≤ 20 μL in volume) of purified total RNA (either bacterial or mammalian) was added to microcentrifuge tubes and left to dry in a laminar flow hood overnight. When the samples were resuspended in disinfectant care was taken to ensure all nucleic acids were exposed to the disinfectant by careful pipetting around the base of the tube.

For purified plasmid: to each tube 10 μL of either disinfectant or control was added and following a suitable incubation period reactions were diluted with 50 μL of nuclease free water to prevent significant further degradation. Reactions were not quenched with a chemical reagent to prevent potential side effects of these chemicals or their interactions with those in disinfectants on the nucleic acid stability. Disinfectants were tested as per manufacturer's instructions where applicable. Samples were all analysed immediately or immediately frozen at –20°C awaiting analysis. When comparing disinfectants/reagents all samples were tested against the same batch of nucleic acids using the exact same method for analysis.

For gDNA or total RNA, to each tube 5 μL of either disinfectant or control was added and then reactions stopped by dilution following the addition of 25 μL of nuclease free water. Disinfectants were applied as per manufacturer's instructions (regarding residence time and working concentration) where applicable.

For time course assays reactions were stopped by dilution with nuclease free water after 0, 5, 10, 15, 30, 45, and 60 minutes. All experiments were completed within a laminar flow hood using filter tips to eliminate any risk of contamination.

Nucleic acid gel electrophoresis

A total volume of 1 μL gel loading buffer (Sigma Aldrich Co. LLC, UK) was added to 4 μL of sample for DNA analysis by gel electrophoresis. DNA samples were run at 120 V for 45 minutes. For RNA analysis, 6 μL of sample was added to 12 μL of RNA denaturing loading buffer without ethidium bromide (Sigma–Aldrich Co. LLC, UK) before heating at 70°C for 10 minutes. Samples were then kept on ice for at least 1 minute. RNA samples were run at 40 V for 40 minutes.

All samples were analysed using a 1% agarose gel, containing 1 × GelRed in 1 × TAE buffer, except where indicated, when instead DNA was analysed using a premade 1% agarose gel (Sigma–Aldrich Co. LLC, UK) and stained in a GelRed solution (1%). Images of gels were taken using a G: Box gel doc system (Syngene) using Genesys software and band intensity determined using ImageJ (Schneider et al. 2012).

Table 1. Summary for the comparison of reagents and disinfectants on nucleic acid degradation.

	NaOH reagent	PCMX disinfectant	LA disinfectant	QAC disinfectant	NaOCl
Impact on transfer of ARGs					
Plasmid degradation					
- DNA Gel electrophoresis	+	++	+	+++	++++
- PCR			++		++++
Mobility of genetic material					
- Chemical transformation			+++		++++
gDNA degradation	++	++	+++	++++	++++
Removal of potentially infectious material					
RNA degradation					
- RNA gel electrophoresis	++++	+++	++++	++++	++++
- qPCR	++++				++++

$^{+}$ (10% reduction), $^{++}$ (11%–25% reduction), $^{+++}$ (26%–50% reduction), and $^{++++}$ (>50% reduction).

Chemical transformation

Samples of purified pUC19 treated with each disinfectant and controls were used to set up a chemical transformation into competent *E. coli* DH5α cells (Invitrogen, ThermoFisher Scientific). To competent cells (50 μL), treated DNA sample (1 μL) was added and the mixture incubated on ice for 30 minutes, prior to heat shock at 42°C for 30 seconds, and recovery on ice for 2 minutes. Cells were recovered in 250 μL super optimal broth with catabolite repression (SOC) media (Sigma–Aldrich Co. LLC, UK) for 1 hour at 37°C before diluting 1 in 10 into sterile media and plating 50 μL onto pre-warmed LB broth with ampicillin (286 μM) plates. Colonies were counted following incubation at 37°C overnight. Data were analysed using a one-way ANOVA with significant differences determined when $P < 0.05$.

PCR analysis

DNA samples (1 μL), post treatment, were analysed by PCR using primers for the ampicillin resistance gene in pUC19 (forward primer: 5′-CCGGGAGCTGCATGTGTCAGAGG-3′ and reverse primer: 5′-ATAATACCGCGCCACATAGC-3′). The predicted PCR product is 489 bp in length. PCR products were purified using a GenElute PCR clean up kit (Sigma–Aldrich Co. LLC, UK) as per the manufacturer's instructions. Purified PCR products were quantified using absorbance at 260 nm.

qPCR analysis

RNA samples (1 μL), post treatment, were analysed by quantitative PCR using the 'one-step' SYBR Green quantitative RT-PCR kit (Sigma–Aldrich Co. LLC, UK) as per manufacturer's instructions. In brief, M-MLV RT was used to transcribe the RNA into cDNA which was then used as a template for the PCR step using SYBER Green Taq. The following primers were used to detect the *ybbw* gene, part of the core set of genes in *E. coli* (forward primer: 5′ -TGATTGGCAAAATCTGGCCG-3′ and reverse primer: 5′ -GAAATCGCCCAAATCGCCAT-3′. The method followed is detailed by (Walker et al. 2017).

Sequencing

PCR products (489 bp) were purified for analysis by sanger sequencing to check for alterations within the sequence due to DNA damage by the products on the plasmid sequence.

Purified PCR products were sent for sequencing at 5 ng μL^{-1} with the forward primer detailed in PCR analysis. Sanger sequencing was outsourced to Eurofins Genomics and analysed using SnapGene software.

Results

Disinfectants and laboratory products were compared for nucleic acid degradation as determined by physical damage and functional damage to either DNA (both mobile genetic element and genomic) or RNA (both bacterial and mammalian). The efficacy of disinfectants and laboratory products across all methods are summarized in a simplified format in Table 1.

Physical Damage to Treated DNA

Purified plasmid (pUC19) was used as a suitable DNA substrate for testing DNA degradation by the disinfectants. Gel electrophoresis allows for the visualization of the purified plasmid pUC19 (2.7 kb) following treatment with the disinfectants and can allow for quantification using band integrations. Gel electrophoresis analysis showed that purified plasmid pUC19 (at 1 μg) demonstrated two bands even when treated with water (negative control). This is likely due to supercoiled versus linear plasmid, with a low level of the plasmid potentially nicked during the experimental procedure itself, Fig. 1. Using gel electrophoresis, the optimal residence time and concentration of a lactic acid (LA) disinfectant could be determined with regards to DNA alteration.

The residence times tested were between 5 and 60 minutes and concentrations between 2% and 10% (as recommended by the manufacturer). DNA gel electrophoresis showed no significant difference in pUC19 degradation, as determined by band position and intensity, following 10 minutes, Fig. 1b and Table S1. Further analysis from 2 to 10 minutes, showed a mild increase in the proportion of linear plasmid up to 10 minutes (Fig. S1, Table S2)

The LA disinfectant did not generate the expected smearing of DNA within gel electrophoresis corresponding to classical degradation of nucleic acids, but instead demonstrated a singular band with a shifted position dependent on concentration of the LA disinfectant, Fig. 1a. One explanation for this observation is that the DNA is dehydrating due to the polar nature of LA, with dehydrated DNA previously shown to alter DNA supercoiling (Lee et al. 1981), so resulting in the DNA running further within the gel. Dehydrated DNA can lead to the denaturation of DNA (Ghoshdastidar and Senapati 2018) and increased dehydration can also make DNA more sensitive to damage by high temperature of UV radiation d(Paunescu et al.

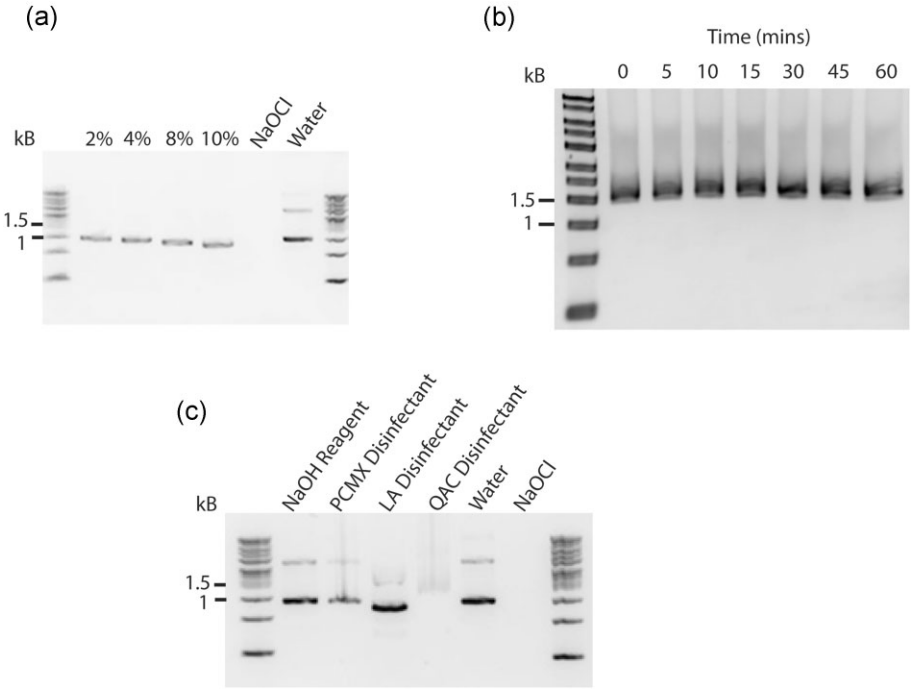

Figure 1. Gel electrophoresis of purified DNA (pUC19) following treatment with disinfectant or equivocal reagent. (a) Effect of different working concentrations of the LA disinfectant on DNA. (b) Time course of the LA disinfectant at 10% effect on DNA. (c) Comparison of disinfectants and equivocal reagents effect on DNA following treatment as per manufacturer's instructions.

2013). We suggest that the lower the band could correlate to greater inactivation of the DNA. The corresponding band also demonstrates a reduction in intensity as the concentration of the LA disinfectant is increased, Table S1. We have therefore concluded that 10% is the optimal working concentration for the LA disinfectant with respect to DNA degradation. This method can also be used to determine efficacy of other disinfectants and here the LA disinfectant is compared to other similar products as per manufacturer's instructions.

Treatment with the QAC disinfectant gave similar results to the positive control (10% NaOCl), Fig. 1c. The NaOH reagent demonstrated no meaningful degradation of pUC19 compared to the negative control (<10% reduction in intensity of either observed band), Fig. 1c. Both bands were observed following treatment with the PCMX disinfectant too; however, both bands show an appreciable reduction in band intensity (17% top band and 47% bottom band). For the LA disinfectant the top band (corresponding to nicked pUC19) showed a 65% reduction in intensity compared to control and is comparable to treatment with QAC disinfectant and the positive control.

Genomic DNA

gDNA can be released following bacterial lysis, a natural consequence following treatment with a disinfectant, and this exogenous DNA can then be taken up by bacteria. For this reason, the efficacy of disinfectants against gDNA is important information.

Interestingly, the results for bacterial total gDNA were different to those for the purified pUC19 plasmid with all concentrations of the LA disinfectant tested (4%, 8%, and 10%) showing complete degradation of up to 2 μg of bacterial gDNA (Fig. 2a). At higher concentrations of 5 or 10 μg of total gDNA minimal degradation was observed compared to the water control, with a 24% and 8% reduction in band intensity, respectively (Fig. S2). Higher concentrations of gDNA could be utilized for experiments if a stronger signal was required for the control. The LA disinfectant (10%) was also shown to degrade Gram-positive DNA (Fig. S3).

The QAC disinfectant and NaOCl degraded the gDNA (at 2 μg) to undetectable levels, whereas the LA disinfectant showed strong degradation with a 44% reduction in band intensity (Fig. 2b). Both the NaOH reagent and PCMX disinfectant showed no reduction in band intensity compared to the negative control following treatment (Fig. 2b).

Functional analysis of treated DNA

Testing the functionality of the DNA following treatment with disinfectants offers insights into their impact on reducing the potential spread of functional ARGs. PCR analysis and sequencing for the ampicillin resistance gene were used to show that the purified plasmid, pUC19, retained sequence integrity following treatment with disinfectants. A 60-minute time course (Fig. 3a), demonstrated a reduction in band intensity for the PCR product across all time points when compared to the negative control.

When comparing disinfectants, all products tested retained DNA of sufficient quality following treatment for detectable levels of PCR amplification of the gene of interest, and this was observed consistently across replicates (data not shown). The positive control, NaOCl, showed a marked reduction in band intensity (96% reduction). There was no reduction in band intensity observed for all other reagents apart from the LA disinfectant which demonstrated a 25% reduction (Fig. 3b, Table S1).

PCR products (489 bp) were purified for analysis by sanger sequencing to check for alterations within the sequence due to DNA damage caused by the products on the plasmid se-

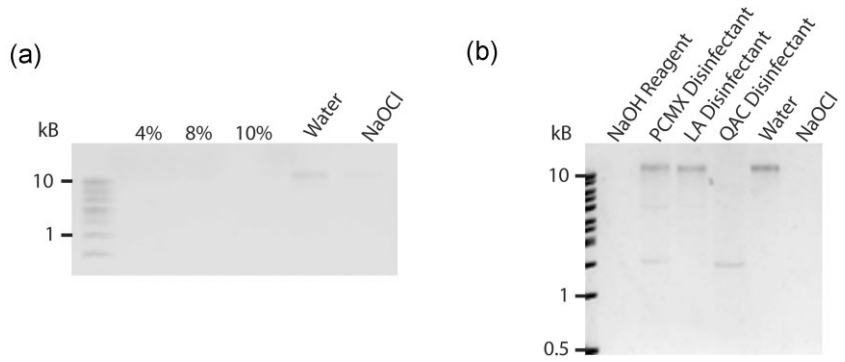

Figure 2. Gel electrophoresis of gDNA (*E. coli* DH5α) following treatment with disinfectant or equivocal reagent. A. Different concentrations of the LA disinfectant between 2% and 10%. B. Comparison of disinfectants and equivocal reagents on gDNA following treatment as per manufacturer's instructions.

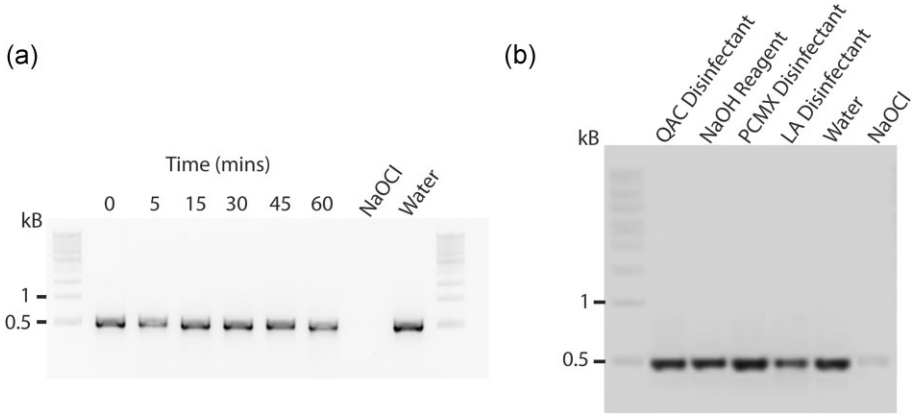

Figure 3. PCR analysis for the ampicillin resistance gene within pUC19 (489 bp product). (a) Time course from 0 to 60 minutes for 10% of the LA disinfectant. (b) Comparison of PCR products using DNA treated with disinfectants and equivocal reagents.

quence. Sequencing data shows that for all the compounds tested no deviations from the known sequence were observed within the DNA template (Fig. S4). Sequencing data were all determined to be of good quality. We suggest that this method is useful in demonstrating that products are not causing mutation inducing damage to the DNA following treatment.

Within molecular biology chemical transformations are frequently used to ensure uptake of plasmids into various *E. coli* strains, with antibiotic selection of successful transformants. Here we describe the use of chemical transformations to investigate the functionality of the plasmids, as a whole, following treatment with products. The results were disinfectant specific, with the LA disinfectant the only product, other than the positive control, showing reduced successful transformants (Fig. 4b). All other products showed no observable effect on the efficiency of chemical transformation, Fig. 4c.

Treatment of the DNA (pUC19) with increasing concentrations of the LA disinfectant (4%, 8%, and 10%) correlated with the corresponding decrease in the number of successful transformants (Fig. 4a and b). A significant difference is observed for treatment with either 8 or 10%, with calculated *P*-values of 0.0037 and 0.0008, respectively. This effect is further exaggerated following 60-minute incubation of the LA disinfectant with the DNA, with all concentrations demonstrating a significant decrease in transformation efficiency, *P*-values of 0.0008, 0.0001, and <0.0001 for 4%, 8%, and 10%, respectively (Fig. S5). One explanation for this observation is the low pH for the disinfectant, however, it is more complex than this as no observable reduction in the measured pH was observed across dilutions of the LA disinfectant. Furthermore, another disinfectant tested with the same active ingredient showed no observable impact on transformation at any concentration (data not shown). Treatment of the DNA with the control NaOCl (10%) completely removes the uptake of functional pUC19 by competent *E. coli* DH5α. To test whether the observation related directly to the DNA damage, or instead the effect of the diluted disinfectant on the process, transformations were completed with the sequential addition of DNA and diluted disinfectants. Using this method comparison of the negative control (water) to the positive control (NaOCl) demonstrated no significant difference to the number of transformants (Fig. S6), so suggesting that the observed effect is due to DNA damage alone. Conversely, we still observed a reduction compared to the water control (*P*-value 0.0028) for successful transformants in the presence of the diluted LA disinfectant. This disinfectant is therefore likely having an impact on the transformation efficiency in addition to any damage to the DNA.

Physical and functional damage to RNA

Many prevalent viral diseases within humans are caused by RNA viruses, where their genetic information is encoded by RNA instead of DNA, examples including noroviruses (Guix et al. 2007)) and HIV (Berkhout and Hemert 1994)). Infec-

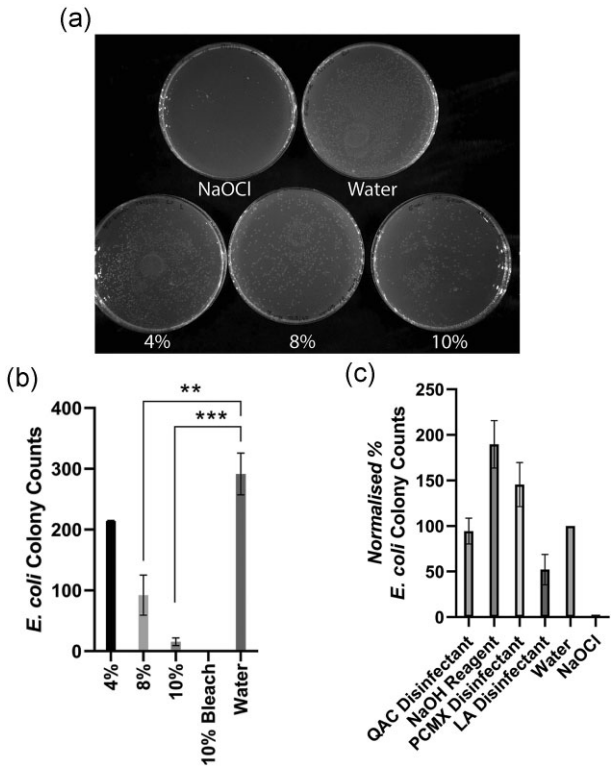

Figure 4. Analysis of the impact on the uptake of DNA (pUC19) by chemical transformation following treatment with disinfectants and equivocal reagents. (a) Plates showing successful transformants for DNA treated with increasing concentrations of the LA disinfectant. (b) Corresponding colony counts for transformations of DNA treated with increasing concentrations of the LA disinfectant. (c) Comparison colony counts for DNA following treatment as per manufacturer's instructions with disinfectants and equivocal reagents.

tious viral RNA genomes are not available within category 1 laboratories, here we have therefore tested mammalian RNA as a proxy for the mammalian RNA viral genomes. Total RNA purified from either bacterial cells (*E. coli* DH5α) or mammalian cells (lymphoblast TK6 cells), were tested at a range of concentrations (1–10 μg). Concentrations of total RNA <10 μg were not consistently detectable by gel electrophoresis, even for controls. Therefore, 10 μg RNA was treated with the products and analysed for signs of degradation by gel electrophoresis.

Optimization trials of the LA disinfectant showed it had a greater effect on RNA degradation at a working concentration of 10% compared to 8% (Fig. 5a and b). This was true for both bacterial RNA and mammalian RNA, with an 77% and 88% reduction in band intensity observed, respectively.

Comparison of different products against bacterial RNA demonstrated complete degradation of RNA to undetectable levels by both NaOCl (10%) and the NaOH reagent (Fig. 5c). Strong smearing can be observed for both the LA disinfectant and the QAC disinfectant, suggesting marked degradation of the RNA. The PCMX disinfectant demonstrates some RNA degradation, marked by reduced band intensity (19% and 32% for 16S and 23S rRNA, respectively), compared to the negative control (water) but this is the least marked effect of all the products tested (Table S1).

Quantitative PCR analysis was used to demonstrate whether the mRNA was functional following treatment, by establishing whether it can still be used as a template for amplification (Fig. 6). No significant difference was observed for QAC disinfectant, PCMX disinfectant and LA disinfectant compared to the negative control of water. Conversely, NaOCl (10%) and the NaOH reagent treated RNA generated no CT value at all, suggesting that the majority of the mRNA is degraded following treatment, which is supported by the gel electrophoresis observations.

Table 2. Absorbance peaks within the 200–300 nm range for 'active ingredients' within disinfectants.

Active ingredient	Absorbance maxima (nm)
Sodium hypochlorite	292 (Nakagawara et al., 1998)
LA	210 (Zhou, Bi and Row, 2011)
NaOH	201 (Tong et al., 2020)
Glutaraldehyde	280 (monomer) (Abay et al., 2019) 235 (polymer) (Abay et al., 2019)
Citric acid	209 (Krukowski et al., 2017)
PCMX	210 (Gudipati and Stavchansky, 1995)

Discussion

Here, we describe the use of common molecular biology techniques using standard reagents available in most research laboratories for testing disinfectants against relevant nucleic acids (plasmids, gDNA, and RNA). It is important to note that absorbance at 260 nm, commonly used to quantify nucleic acid concentrations is not applicable in this context with many disinfectants demonstrating absorbance maxima between 200 and 300 nm (Table 2). These methods can be used, as shown in this study, to suggest optimal residence times and working concentrations for the degradation of nucleic acids. There are inherent difficulties in quenching reactions when determining the impact of a disinfectant on nucleic acid integrity over time. These include the fact that the addition of chemicals has the potential to interact with the disinfectant and cause side products capable of worsening the observed degradation, dilution may not be sufficient to prevent all further degradation particularly for disinfectants with strong activity and introducing a freezing step could introduce DNA shearing from the freeze-thaw process. Here we have suggested the use of water to dilute the reaction, as when tested we observed a reduction in the rate of degradation for the LA disinfectant following dilution (Fig. S1). This may not be the case for all disinfectants tested and therefore we suggest coupling this step with flash freezing samples if further degradation is observed post dilution. It is important to note that the optimal residence times for antimicrobial activity of the disinfectant may differ from the optimal residence time for nucleic acid degradation, if both are stated within the packaging for the disinfectant it allows for alternative application dependent on the environment for use. Alternatively, companies could adapt experiments to determine whether any nucleic acid degradation is observed within the antimicrobial residence time.

The degradation of nucleic acids within a healthcare environment is of key importance to reduce multi-drug resistant infections, particularly within the healthcare setting. Yet, Stoufer et al. demonstrate the limitations of common disinfectants to degrade a variety of nucleic acids (ssRNA, eukaryotic DNA, and PCR products) by monitoring downstream quantitative PCR (Stoufer et al. 2023). The ability of UV light to

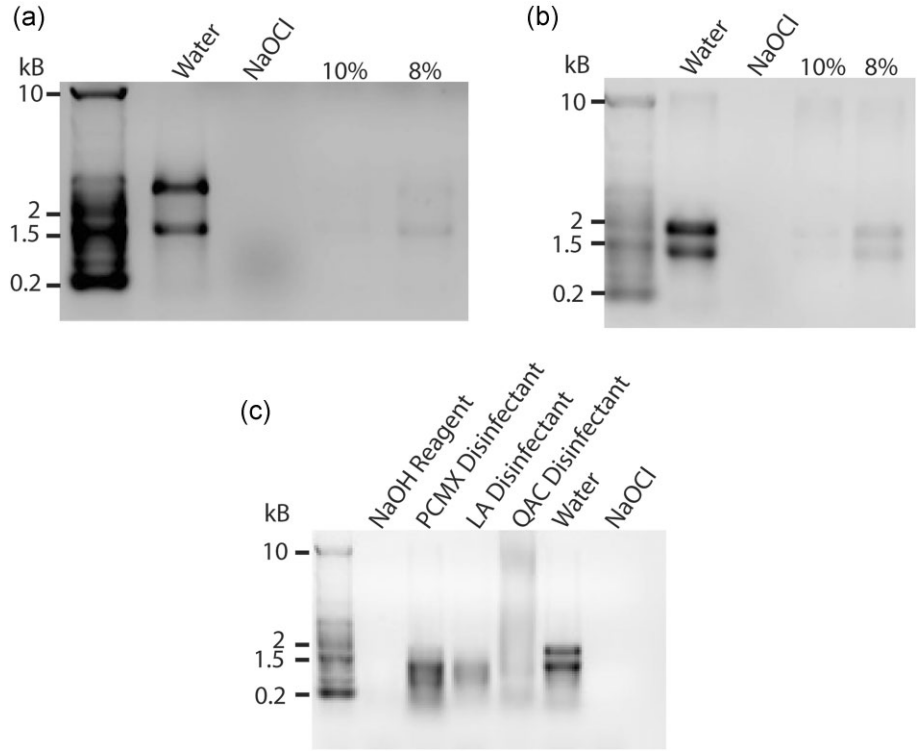

Figure 5. Gel electrophoresis of RNA (*E. coli* DH5α or TK6 lymphoblast cells) following treatment with disinfectant or equivocal reagent. (a) Comparison of the LA disinfectant concentrations and controls for degradation of mammalian RNA. (b) Comparison of LA disinfectant concentrations and controls for degradation of bacterial RNA. (c) Comparison of disinfectants and equivocal reagents on bacterial RNA following treatment as per manufacturer's instructions.

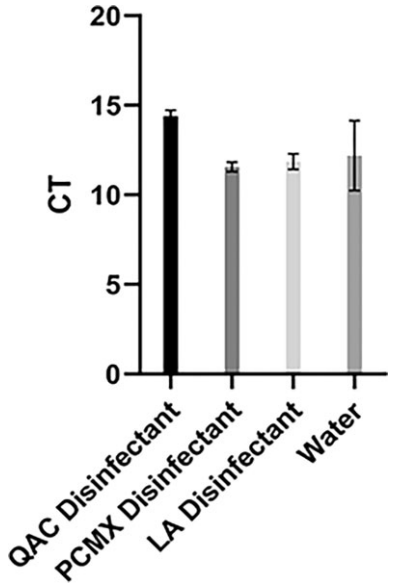

Figure 6. Comparison of the functionality of bacterial mRNA following treatment with disinfectants and equivocal reagents as per manufacturer's instructions using qPCR analysis for the *E. coli ybbw* gene.

damage the molecular bonds within DNA has been shown to reduce contamination by MRSA, VRE, and *Clostridium difficile* on high-touch surfaces (Nerandzic et al. 2010). Yet, it is impractical to use UV light to decontaminate the vast number of surfaces within healthcare settings. NaOCl, a common surface disinfectant, showed the most promising results here regarding nucleic acid degradation. This observation is supported by research published in 2019 suggesting free chlorine is an effective method for inactivating extracellular chromosomal and plasmid-borne DNA (Zhang et al. 2019). In 2020, however, Jin et al. reported that chlorine disinfection promotes the exchange of ARGs by transformation due to various bacterial species demonstrating resistance to NaOCl (Jin et al. 2020). Testing and reporting the activity of all disinfectants against nucleic acids would allow appropriate selection of disinfectants for use within the healthcare system. With further research required into what impact this has on the reduction in the contamination of these environments with multi-drug resistant bacterial species. The importance of cell-free DNA as a source of ARGs within wastewater treatment has been previously highlighted (Leiva et al. 2021, Nguyen et al. 2021, Hutinel et al. 2022), with a 0.12 log increase in cell-free ARGs observed following 25-day storage of disinfectant treated water (Zhang et al. 2018).

There is currently a dearth of literature investigating the impact of disinfectants or cleaning reagents on nucleic acids, although interest in this area seems to be increasing. One explanation for this is that it is non-trivial to find methods to analyse DNA degradation by a variety of different active compounds with different modes of action. Most research focuses on DNA damage, but Zhang *et al*. has investigated the effectiveness of disinfectants (in isolation or combination) against SARS-CoV-2 RNA. Interestingly, they note that detectable levels of the RNA were present even after 60 minutes exposure with a quarternary ammonium compound (QAC) disinfectant (Zhang et al. 2023). This matches our findings that a QAC containing disinfectant did not result in complete degradation of the RNA following 10-minute incubation, with only partial

degradation observed via RNA gel electrophoresis and no impact on qPCR analysis, Figs. 5c and 6. Conversely, the NaOH reagent demonstrated strong activity against RNA. This could be due to the additional hydroxyl group at the C2′ position of the ribose within RNA which can be deprotonated in strongly alkaline conditions. Here we have demonstrated activity against mammalian and bacterial RNA, however given the structural variation observed across viral RNA genomes (Davis et al. 2008) further work investigating the impact of disinfectants against purified viral RNA would offer further insights.

Previous comparison of reagents for DNA degradation within a laboratory setting to reduce PCR contamination, demonstrated that the NaOH containing reagents showed a dose and time-dependent reduction of amplifiable DNA, as assessed by PCR (Fischer et al. 2016). In this study, however, we observed minimal impact (<1% reduction) on the amount of amplifiable DNA following treatment with the NaOH-containing reagent (Fig. 3b). This demonstrates the importance of using several techniques to establish the efficacy of a reagent at nucleic acid degradation as the same compound showed complete degradation of RNA (Fig. 5c).

Plasmids are autonomous DNA molecules capable of self-transmission between cells and are therefore important with regards to the emergence of antimicrobial resistance (Rozwandowicz et al. 2018). High copy number plasmids, such as pUC19, can reach high concentrations within their bacterial hosts and provides a method for monitoring the impact of disinfectants on plasmid uptake. None of the disinfectants/reagents tested were able to fully degrade the plasmid pUC19. Many disinfectants contain chemicals, which are dangerous to health and therefore working concentrations are kept low, it could be that the concentrations of these chemicals required to completely remove plasmid DNA are too high for reasonable use. By analysing the impact that the disinfectants have on plasmid uptake by bacteria, this could help illuminate which disinfectants are likely to reduce the spread of mobile genetic elements and associated ARGs. In this study, the LA disinfectant appeared to have an impact on the transformation process itself as well as impacting the DNA quality. Further investigation is needed to demonstrate the *in vitro* effects are also observed *in situ*.

Current literature within this field predominantly demonstrates activity of the reagent tested against one type of nucleic acid or using one analysis method (Esser et al. 2006, Champlot et al. 2010, Fischer et al. 2016, Stoufer et al. 2023, Zhang et al. 2023). Reassuringly, we show that results are frequently comparable across different analysis methods for different products, with the positive control of bleach (10% NaOCl) routinely producing the clearest results. Within the product range tested in this study, the QAC disinfectant and LA disinfectant were also reproducibly more active against nucleic acids across all analysis methods. This can be linked to the known precipitation of nucleic acids by QACs such as dequalinium acetate (Hugo and Frier 1969), and the depolymerization of nucleic acids by acid hydrolysis (WEBB 1958, Huang et al. 2012, Lowenthal et al. 2019). PCMX is a chlorinated phenolic compound with widespread uses, particularly as a disinfectant, with proven activity against several microbial species (Arbogast et al. 2019). In this study the PCMX disinfectant, a commonplace disinfectant within households, showed the least degradation of all nucleic acids tested.

By analysing the effect of the product across several parameters, manufacturers can produce clearer claims within their marketing, which will better inform use and help reduce the spread of infectious agents, mobile genetic elements and antimicrobial resistance genes. Here, we have focussed on easily accessible methods and materials for research laboratories to test their products. Moving forward the standardization of the methodologies used and the subsequent transparency regarding results will offer a pathway for the potential regulation of cleaning products activity against nucleic acids.

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Supplementary data

Supplementary data is available at *JAMBIO Journal* online.

Conflict of interest: This work was supported by a cleaning solution manufacturer which provided the lactic acid disinfectant for testing. All authors declare no further conflict of interest.

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Author contributions

Rachael C. Wilkinson (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing), Kirsty Meldrum (Data curation, Investigation, Methodology, Writing – review & editing), Caitlin J. Maggs (Investigation, Methodology, Writing – review & editing), Nerissa E. Thomas (Formal analysis, Writing – review & editing), Bethan R. Thomas (Project administration, Writing – review & editing), Natalie De Mello (Project administration, Writing – review & editing), and Naomi Joyce (Funding acquisition, Writing – review & editing)

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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