Original Article

Safe disposal of *E. coli* DH5α strain from liquid broth and solid substrate

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ABSTRACT

Healthcare waste contains potentially infectious agents that represent risks for human health and the environment. Many techniques can be used to decontaminate these infectious wastes and make the management process safer and less costly. In the present study, various chemical disinfectants were used to decontaminate concentrated *E. coli* DH5α strain bacterial broth used in laboratories, as well as gauze contaminated with these bacteria. Dose-response statistical regressions were performed after multiple comparisons using the Kruskal-Wallis test with Nemenyi post-hoc. Fifty percent lethal concentration (LC_{50}) for E. coli DH5α was 0.0586% for sodium hypochlorite (NaOCl), 0.0243 % for hydrogen peroxide (H_2O_2), 0.0161 % for formaldehyde, 12.20 % for ethanol, and 0.00401% for quaternary ammonium (BADAC-DDAC). When used to treat solid waste, all the chemicals used were able to completely disinfect the gauze when the concentration of $5\times LC_{50}$ was used. This research highlights the adoption of biocides as efficient, low-cost alternative healthcare waste treatment options.

KEYWORDS

infectious waste,

healthcare waste,

chemical decontamination,

waste treatment.

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INTRODUCTION

Inadequate management of infected waste represents a challenge for managers due to impacts on health and the environment (**Brunello *et al.* 2011**, **Shanmugasundaram *et al.* 2012)**. This situation has been aggravated by the growing generation of healthcare waste (HCW) associated with the continuous increase in healthcare complexity and increased use of disposable materials (**Cheng *et al.* 2009**, **Hossain & Alam 2013, Jovanović *et al.* 2016, Ream *et al.*, 2016**). **Ream *et al.*, 2016** showed that accidents are frequently registered for health waste workers, representing a high potential risk. Thus, alternatives for HCW treatments are necessary to make the management waste process safer and more cost effective.

HCW treatment costs represent values between 0.74-3.93 US$ Kg^{-1} for hazardous wastes in countries including the USA, Brazil, Italy, and Japan (**Barbosa & Mol 2018, Vaccari *et al.* 2018, Windfeld & Brooks 2015, Miyazaki & Une 2005**). Therefore, determining and validating new techniques that increase efficiency and have less significant costs is of great value, both to healthcare and to the environment.

Healthcare wastes including infectious agents may represent the risk of infection and should be treated where they were generated, as recommended by the World Health Organization (**WHO 2018**). Many techniques can be applied to

decontaminate infectious wastes, including incineration, plasma, autoclave, or disinfection by chemicals **(Gul *et al*. 2020**). Furthermore, a range of chemicals are currently used as decontamination agents, such as calcium or sodium hypochlorite (NaOCl), alcohol, quaternary ammonium compounds, ozone, peracetic acid, hydrogen peroxide and organic acids (**Rutala & Weber 1997**).

The NaOCl disinfectant is the active compound of the commonly used cleaner bleach and is among the most effective chemicals used for instant microbiology decontamination in hospitals and biological laboratories due to its low cost and efficacy (**Pereira *et al*. 2015**). This compound germicide capacity results from ability to dissolve free gaseous chlorine, the germicide agent. In addition, NaOCl degrades organic material and reacts with lipids through saponification (**Guida 2006**). It is believed that NaOCl acts by inhibiting key enzymatic reactions by protein denaturation and nucleic acid inactivation in bacteria, viruses, and fungi (**Kalil & Costa 1994**). Temperature, solvent concentration, and pH can affect NaOCl composition and activity. Light can render it inactive, reducing disinfection potential According to the United States Environmental Protection Agency (EPA), "no sub-chronic or chronic studies on sodium and calcium hypochlorite are needed due to their simple chemical nature and structure. In the presence of oxygen, these compounds react easily with organic matter and convert readily into sodium chloride (table salt) and calcium chloride (road salt). Widely used in disinfecting water supplies for nearly a century, hypochlorite has been proven safe and practical to use without consequence to human health" (**USEPA 1991**).

Ethanol is regularly used as a disinfectant agent, not only in laboratory routine but also for everyday life, from hospital to personal disinfection. It is considered bactericidal, fungicidal, and virucidal, acting in protein denaturation, but lacks sporicidal activity; for this reason, it is not recommended for sterilization (**CDCP 2008**). Different concentration of ethanol is effective on different microorganism; however, the recommended concentration for broad-spectrum decontamination is 70% in either formulation, liquid, or gel. Currently, during the coronavirus (COVID-19) pandemic, consumers are frequently using both liquid and gel ethanol, demonstrating the necessity of validating the effectiveness of these compounds against various microorganism commonly encountered in everyday life (**WHO 2020, Kampf *et al*. 2020**).

Since formaldehyde is bactericidal, fungicidal, virucidal, and sporicidal, it can be used for decontamination and sterilization in liquid as well as gaseous states. Formaldehyde inactivates microorganisms by alkalazing the amino and sulfhydryl groups of proteins and the nitrogen ring atoms of purine bases. The biggest risk resulting from the regular use of formaldehyde as a disinfectant agent is toxicity to humans, it is potentially carcinogenic. The Occupational Safety and Health Administration (OSHA) limits formaldehyde exposure to an 8-hour time-weighted average concentration of 0.75 ppm (**OSHS 2020**).

Hydrogen peroxide (H_2O_2) is an efficient oxidizing agent of organic matter. A concentration of 3-6% can eliminate most bacteria. Concentrations as high as 10-25% kill microorganisms and spores (**Murray *et al*. 2017**). The antimicrobial action of H_2O_2 results from the formation of free hydroxyl radicals, which attack the lipid cytoplasmic membrane, DNA, and cellular components essential for life (**Matos *et al*. 2010**). Since H_2O_2 is effective against all life forms only in high concentrations, it is not usually used to disinfect rather than sterilize.

Benzyl alkyl dimethyl ammonium chloride/dodecyl dimethyl ammonium chloride (BADAC/DDAC) is quaternary ammonium and is the active compound of a household cleaning disinfectant. The label concentration is 0.45% and

the recommendation is to use it undiluted to "kill 99.9% of germs and bacteria" (product label). Generally, quaternary ammonium is fungicidal, bactericidal, and active against lipophilic viruses; however, it is not active against hydrophilic viruses and spores, and it is not effective against mycobacteria.

To confirm the decontamination effects of chemicals, such as those described above, current recommendations are to test the dose-response of the chemical when applied to a known microorganism commonly encountered in healthcare facility routines. For this paper, we tested *Escherichia coli*, Gram-negative bacteria naturally found in the intestinal tract of animals. The strain DH5α is currently used in biology, molecular biology, biotechnology laboratories and industries in the cloning and expression of exogenous proteins. They are classified as agents of risk class 1 (**NIH 2020**), which means it poses low individual or community risk and do not pose any risk to healthy adult animals.

Although studies focused on disinfection components have advanced in the last century, each active component has a mode of action that makes them unique for a decontamination methodology. The search is ongoing for sustainable processes that produce less waste and less possible inequity. The goal of the present study is to analyse the various concentrations of NaOCl, ethanol, H_2O_2, formaldehyde, and BADAC/DDAC necessary to decontaminate *E. coli* DH5α strain in bacterial broth. Also, we validate the optimal concentration for these disinfectants on a solid substrate. Moreover, this study evaluates the time-dependence for NaOCl effect, as well as the role of light was evaluated at intervals over the course of 8 weeks. Decontamination effectiveness of liquid and gel ethanol on topical hand application with and without gloves was also tested.

MATERIALS AND METHODS

Determination of free chlorine

Free chlorine in hypochlorite was determined using volumetric method (**ANVS 2019**). following the regulation by a certified laboratory (FUNED report# 699.1P.0/2019). The label was marked 10-12%; however, the concentration found was 8.63 ± 0.4. The hypochlorite concentrations in all graphs were adjusted considering this value.

Viability assay

E. coli bacteria strain DH5α was grown at 37°C in LB (Lysogenic broth) medium to an OD_{600} between 0.4 and 0.6 (2-3 hours). The bacteria were kindly donated by Molecular Toxicology Laboratory (FUNED, Belo Horizonte, MG). The disinfectants and the respective concentrations used are summarized in Table 1.

Table (1) Type of disinfectant and their respective concentration used.

Disinfectant	Stock Solution (%)	Concentrations (%)
NaOCl	10	5, 1, 0.5, 0.1, 0.05, 0.01
H_2O_2	30	1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001
Ethanol	96	96, 85, 70, 55, 40, 25, 10
BADAC/DDAC	0.45	0.45, 0.3, 0.1, 0.05, 0.01, 0.005
Formaldehyde	37	30, 20, 10, 1, 0.1, 0.01, 0.001

To preserve the amount of bacteria in each well, for dose-response experiments, 150 µL bacteria were plated in each well of a 96 wells plate and centrifuged at 3500 rpm for 5 minutes at room temperature in a plate rotor centrifuge. The supernatant was discharged, and bacteria were incubated with different disinfectants concentrations diluted in LB medium, made with 15 µL of the 10× disinfectants (pre-diluted in water) diluted in 135 µL of LB medium to make 150 µL of total incubation volume. Concentrations higher then 10% of stock solution (table 1), were diluted directed into LB medium (if needed) to the required concentration, the volume was always kept in 150 µL. After 30 minutes at 37°C incubation, the plate was centrifuged twice at the same conditions, and washed with equal volume of LB medium to remove the disinfectant residue. Then, 150 µL of LB medium containing resazurin sodium salt (100 µM, Sigma; diluted from a 10× stock solution), for colorimetric assay, was added and incubated at 37°C. Since most of the disinfectants were tested in the same 96-wells plate, a microseal´s film was used to avoid cross-contamination (i.e. a disinfectant in aerosol or gaseous form that could kill bacteria in another well).

Resazurin (100 µM) is a non-toxic, non-fluorescent, and cell-permeable dye that is reduced to resorufin, a highly-fluorescent, reddish compound in live cells bacteria [26]. The colour change in the control condition determined the incubation time, which varied in bacteria between 30-60 minutes.

To test disinfection time-dependence, a fresh-diluted 0.1% NaOCl was incubated with bacteria for various intervals (5, 15, 30, 45, 60, and 120 minutes) in microtubes. To stop reaction, bacteria were centrifuged 3500 rpm for 5 minutes at 4°C and washed once with LB medium. They were transferred to a 96 wells plates and incubated with resazurin, as described above.

To evaluate the effect of the light on hypochlorite degradation, 20 mL of NaOCl was incubated in flasks of clear or amber glass in a KBF LQC 240 constant climate chamber with Light Quantum Control for stability tests. The samples were kept at 25°C and 75% humidity and exposed to 100.00 LUX of white light and 16.66 Wh/m3 of UV light simulating 8 weeks of exposure to a standard room lamp. Samples were removed and diluted to 0.1% concentration as described previously for dose-dependent experiments; sample viability was tested the same day they were removed. The experiment was made in triplicate.

For all experiments, optical densities at 570 nm and 600 nm in a microplate reader (Biotek, Winooski, Vermont, USA) measured if the resazurin was reduced or not. The relationship between these two measurements is directly related to the bacteria death through equation 1 (**Lancaster & Fields 1996**):

$$\%\ \text{Death} = \{100 - [A570 - (A600 * R0)\ \text{treated}] / A570\ (A600 * R0)\ \text{control}\} * 100$$

Eq. (1)

Where A is the absorbance at a given wavelength (570 or 600 nm) and R0 is the correction factor for a given solution. R0 was calculated using the equation R0 = CA570 / CA600, where CA is the difference between the solution in the presence and absence of resazurin in each wavelength.

The dose-response curves were fitted with the equation of ligand ligation through equation 2:

$$f(x) = (Bmax * [X]) / (LC_{50} + [X]) \qquad \textbf{Eq. (2)}$$

Where Bmax is the maximum proportion of death, [X] is the disinfectant concentration and LC_{50} is the lethal concentration, which kills 50% of the bacteria.

Topical ethanol decontamination

Ethanol is used in a topical application for cleaning and disinfection in the household medical and personal care. Due to the spread of COVID-19

pandemic, the use of 70% ethanol liquid and gel for disinfection is constant in the general population. Therefore, we tested lab-made 70% liquid ethanol (diluted in distilled water) and 2 brands of commercial gel ethanol for DH5α bacteria decontamination (since they are registered trademark, we do not have authorization from factory we cannot publicized the brand). For these experiments, sterile gloves were carefully put on in the biological safety cabinet and touched to an LB-agar plate. The same finger was submerged into LB-containing bacteria, allowed to dry for 2 minutes, and touched to an LB-agar plate. The same finger was then submerged in the ethanol and dried for 2 additional minutes by scrubbing it against the back of the contralateral finger. It was touched to the LB-agar plate. The same procedure was followed without gloves after the hands were washed with antiseptic soap, sprayed with ethanol, and allowed to dry completely in the biological safety cabinet.

The plates were incubated 37°C overnight. The following day, the growth was qualitatively observed. Some of the areas where the plate had been touched were cloudy, and it was difficult to evaluate whether the cloudiness indicated growth or whether it was glove powder. To determine whether the substance was bacteria or powder, a sterile tip was scratched on the area and placed in liquid LB medium for 5 hours at 37°C in the shaker at 200 rpm OD_{600} was then measured.

Validation

To validate disinfectant efficacy on solid substrate, we attempted to simulate daily-use conditions by using commercial reagents (when possible) and diluting the disinfectants in sterile water, instead of LB medium. A piece of 8 cm of gauze (0.65-0.75 g) was cut and autoclaved in a petri dish. Bacteria *E. coli* DH5α (1 mL) grown to OD_{600} between 0.45-0.60 were poured over and absorbed by these gauzes and incubated at 37°C for 30 min. Post incubation, these gauzes were submerged in 10 mL of each disinfectant diluted in sterile distilled water at concentrations equivalent to $1\times LC_{50}$, $3\times LC_{50}$, and $5\times LC_{50}$, and incubated at room temperature for 30-40 min under room light and agitation (80-100 rpm). Then, 100 µL of the supernatant was inoculated in 10 mL LB medium and kept in a shaker overnight (200 rpm at 37°C). The following day OD_{600} was measured.

Statistics

For dose-response and NaOCl kinetics, the analysis was done for, at least, three independent experiments performed in triplicates. Differences between groups were verified using the Kruskal-Wallis test, for non-parametric data, with Nemenyi post hoc. Normality was tested using the Shapiro–Wilk test. P-value was evaluated using a confidence level of 95%. Dose-response statistical regression indicates the association between the independent and the dependent variables. Weibull, Log-logistic, and lognormal models were adopted through package "drc" for the software R (version 3.4.2), and the best fit model was presented (**Ritz *et al*. 2015**).

Topic ethanol experiments were performed at least 3 times for each condition. Solid waste experiments represent an average of at least 5 independent experiments, and the significance in difference between experimental and control condition were tested by Student t-test.

RESULTS

Fig 1A shows the dose-response curve for NaOCl, fitted by the Weibull model. The data indicate that incubation of *E. coli* DH5α bacteria broth with freshly diluted NaOCl for 30 min killed bacteria with an LC_{50} of 0.0586 % [0.0359 - 0.0814]. However, if the bacteria were incubated in the disinfectant longer, efficiency was improved, as shown in Fig 1B. This graph shows that the bacteria were incubated for various intervals in NaOCl 0.1%. This concentration was chosen because it is almost twice the LC_{50} and is not yet the concentration of

saturation. The same results were achieved after 1 and/or 2 hours of incubation, suggesting that 1 hour reach the maximum time to kill in this concentration. It was also tested how light interferes with disinfection by incubating NaOCl in flasks transparent or amber in a Light Quantum Control chamber, which simulates regular visible light. As shown in Fig 1C, the activity in the transparent flask decreased faster than in the amber flask. After 4 weeks, activity in the transparent flask reduced by 21 %, and activity in the amber flask reduced by 9 % compared to the control condition. After 8 weeks, activity in the transparent flask decreased 79%, and activity in the amber flask decreased 17% compared to the control condition. The experiment represents the average of triplicate.

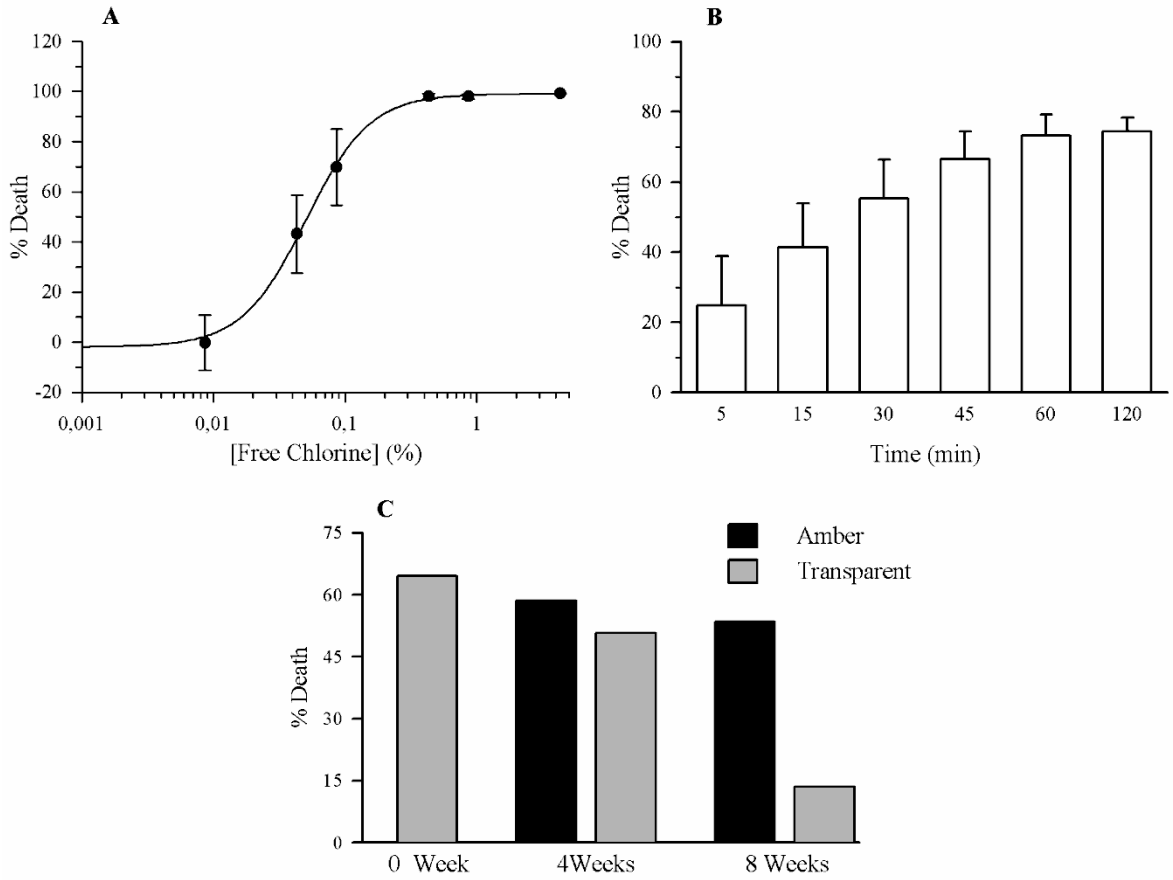

Fig 1. Decontamination of *E. coli* DH5α strain by NaOCl. (A) The dose-response curve shows LC50 of 0.0586%. (B) Curves represent average ± SEM of death versus time of incubation. (C) Degradation of NaOCl by light.

The effect of the H_2O_2 has been showing in Fig 2A. H_2O_2 killed bacteria with LC_{50} 0.0243% [0.00965 - 0.0388], fitted by Log-logistic model. Although NaOCl and H_2O_2 are both unspecific, it seems that the oxidant power of the H_2O_2 against organic matter is stronger: it can be diluted 10× more than NaOCl and produce the same effect. Interestingly, H_2O_2 is used to induce oxidative stress in eukaryotic cells. However, the concentrations used for such experiments are between 1 and 100 μM that are at least three orders of magnitude below those used to kill bacteria in our experiments, which varies between 3 and 300 mM (0.01-1%).

Formaldehyde killed bacteria with LC_{50} 0.0161 % [0.00260 - 0.0295], as presented in Fig 2B, fitted by the Weibull model.

BADAC-DDAC killed bacteria with LC50 0.00401 % [0.00218-0.00582], fitted by Log-logistic model (Fig 2C).

Ethanol killed bacteria with LC50 12.20 % [7.53 - 16.88], fitted by the Weibull model (Fig 2D).

Ethanol is commonly used in daily practice in a concentration of 70 %. The CDC recommends using ethanol above 50 % v/v; below this concentration, they claim the germicidal potential drops sharply (**CDCP 2008**).

The dose-response results are summarized in Table 2, as well as the concentrations equivalent to $1\times LC_{50}$, $3\times LC_{50}$, and $5\times LC_{50}$, used in solid substrate experiments.

Table (2) Summary of the concentrations for validation of the dose-response experiments.

Disinfectant	**LC_{50}**	**Dilution (stock/LC_{50})**	**Concentration (%) $1\times LC_{50}$, $3\times LC_{50}$, $5\times LC_{50}$**
NaOCl	0.0586% [0.0359-0.0814]	1:145	0.05, 0.15, 0.25
H2O2	0.0243% [0.00965-0.0388]	1:1250	0.025, 0.075, 0.125
Formaldehyde	0.0161% [0.00260-0.0295]	1:2055	0.015, 0.045, 0.075
Ethanol	12.20% [7.53-16.88]	1:7.4	15, 45, 75
BADAC/DDAC	0.0040% [0.0022-0.0058]	1:112.5	0.004, 0.012, 0.02

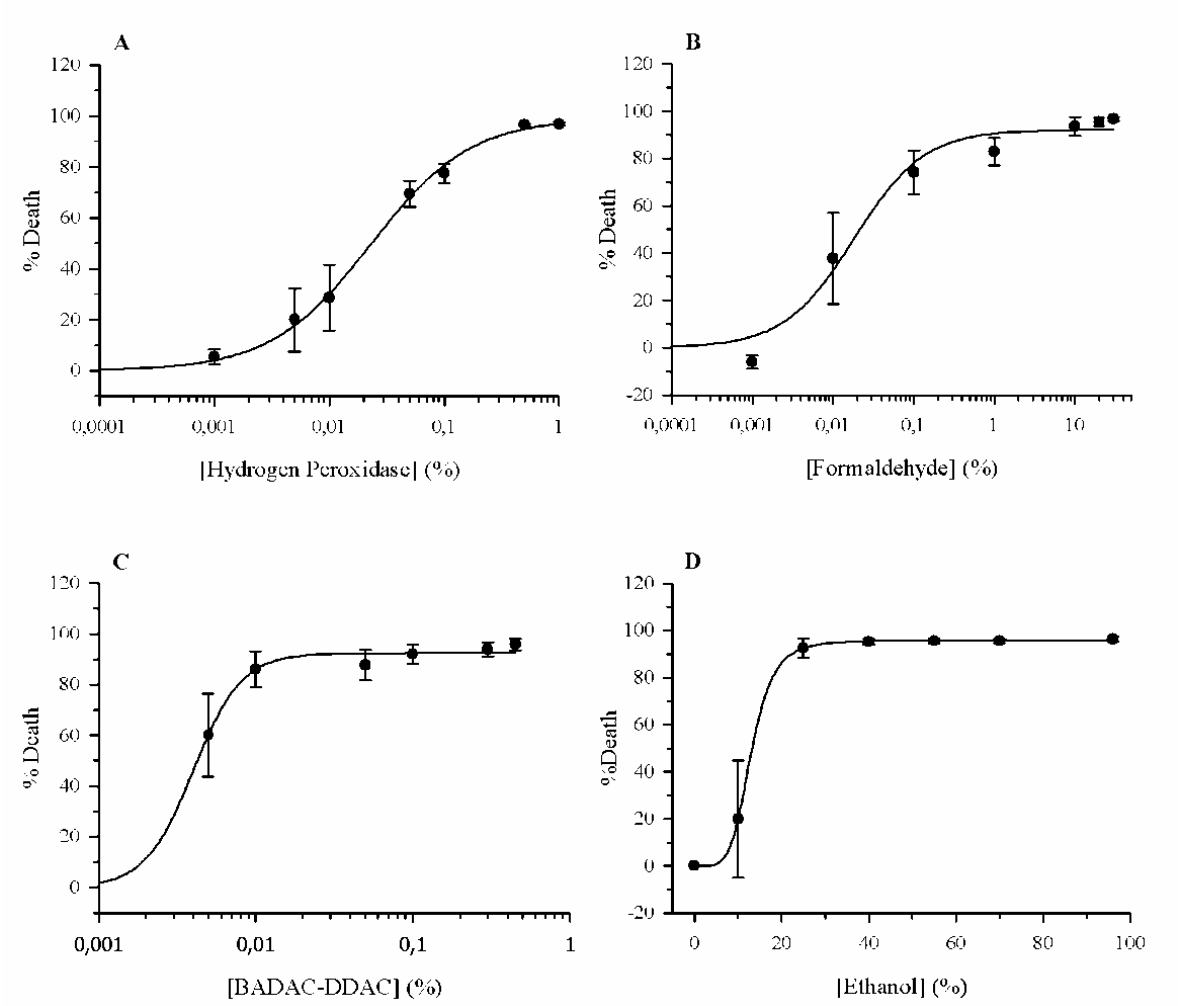

Fig 2. Dose-response effect of E. coli DH5α strain decontamination. (A) Hydrogen peroxide. (B) Formaldehyde. (C) BADAC-DDAC. (D) Ethanol.

Summary of LC_{50} found in the dose-response experiments (confidence intervals in brackets), dilution of the disinfectant LC_{50} from the stock solution, and concentrations used in the solid waste

decontamination, equivalent to 1×LC_{50}, 3×LC_{50}, and 5×LC_{50}. The use of topical ethanol to decontaminate solid substrates, such as gloves or even hands, was tested.

The results were very consistent and showed that 70 % ethanol liquid or gel was able to decontaminate the fingers with and without gloves very efficiently, as shown in Table 3.

Table (3) Hands with or without gloves are efficiently disinfected by liquid or gel 70% ethanol.

Control		**70% Ethanol – with/without gloves**		
+	-	Liquid	Gel 1	Gel 2
1.12±0.067	0	0/0	0/0	0/0

OD_{600} measured after tip scratched in LB-agar was left to grow for 5 hours in LB media.

It was validated the effectiveness of the LC_{50} found in dose-response experiment on solid substrate, simulating the real utilization of the disinfectant in household or hospital. The results are shown in Fig 3. In the control condition, gauze contaminated with bacteria *E. coli* DH5α was incubated in water without disinfectant. Another control (not shown) was made in which gauze was not contaminated with bacteria, but 1 mL of LB medium was poured over the gauze also incubated in water. In this negative control no growth was observed. Since the samples were incubated overnight, most of them reached the logarithmic growth. Bacteria growth were observed in all LC_{50} concentrations and differed significantly from the control ($p<0.05$), except with the ethanol 1×LC_{50} ($p=0.306$). It was also determined that 3×LC_{50} was effective for all chemicals but formaldehyde and BADAC/DAAC. The 5×LC_{50} was effective for all chemicals.

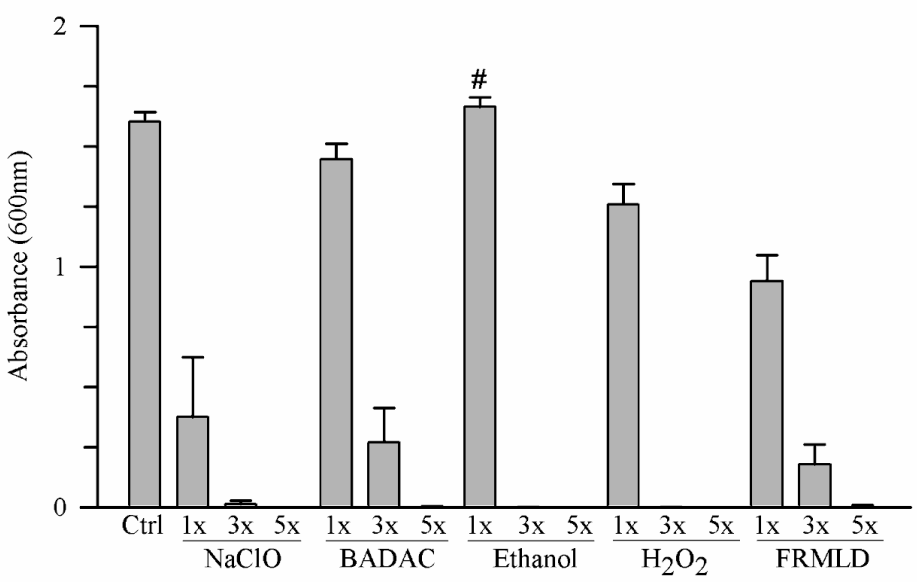

Fig 3. Efficacy of solid substrate disinfection. Concentrations were equivalent to 1xLC_{50}, 3xLC_{50}, and 5xLC_{50} of the disinfectant agent (for values see Table 2). All disinfectant concentrations tested were statistically different from the control (bacteria in water), except 1xLC_{50} ethanol (#).

In the dose-response experiment, the most effective disinfectant was formaldehyde, which was effective even in most diluted concentration (1:2055). On the solid substrate, formaldehyde had a significant reduction in the bacteria growth at 1×LC_{50}. It had a stronger growth inhibition at 3×LC_{50}, but it was only completely effective at 5×LC_{50} (0.075%). Moreover, formaldehyde is toxic to humans; therefore, other disinfectants should be preferred.

DISCUSSION

These findings complement **Rutala and Weber´s (2016)** consideration of disinfections used to clean heat-sensitive items that cannot support sterilization or for which sterilization methods are not viable (**Rutala and Weber´s 2016**). In such cases, the use of products such as formaldehyde, hydrogen peroxide, sodium hypochlorite, BADAC/DDAC, and ethanol may be useful, as demonstrated in this study.

Formaldehyde has been used since the late 1880s for fumigation (**Lach 1990**). It is considered the gold standard for gaseous decontamination of laboratories, animal facilities, and animal farms. The disadvantage of formaldehyde is mainly related to the human health hazard and environmental impact, which has motivated the reduction of use and a search for safer alternatives (**Quantun & Koenen 2011**). For animal facilities, vaporized hydrogen peroxide has been validated to replace formaldehyde (**Sloetjes & Draaijir 2011**).

A study performed by **Motta *et al*. (2018)** focused on decontaminating E. coli using sodium dichloroisocyanurate (NaDCC) (**Motta *et al*. 2018)**. Microorganisms capable of proliferating in the waste bin were not able to grow in the presence of NaDCC, showing that the proliferation of tested bacteria was completely inhibited. This study found some chemicals with the ability to decontaminate *E. coli* were effective in controlling and/or inhibiting microbial proliferation and support NaDCC possible use in the treatment of HCW, biotechnological laboratories, and industries to control the spread of contamination.

One study focused on coronavirus inactivation, published by **Kampf *et al*. (2020)**, suggested efficient disinfection of surfaces using 62 – 71% ethanol, 0.5% H_2O_2, and 0.1 % NaOCl within less than 1 min, and less effective results for 0.05–0.2% benzalkonium chloride and 0.02% chlorhexidine digluconate (**WHO 2020, Kampf *et al*. 2020)**.

When compared with various HCW treatment techniques, as a microwave process, **Huang & Sites (2010)** showed that material inoculated by 5 log10 *E. coli* O157:H7 achieved complete inactivation at temperatures above 70°C for more than 1 min (**Zimmermann 2017**). He also concludes that more sophisticated microwave technologies are effective in rendering biohazardous waste inactive (**Zimmermann 2017**); however, he emphasizes the high cost of keeping this technology in operation. The incineration process is considered one of the biologically safest methods for HCW treatment (**Blake *et al*. 2008, Pils *et al*. 2019**); however, inactivation of the waste in loco is desirable to avoid accidents during transportation.

This research showed a simple low-cost procedure using ordinary chemical disinfectants with enough efficiency to decontaminate concentrated bacterial broth from biotechnological industries and laboratories, as well as HCW infected by *E. coli*. We understand the reality of low-income countries and hope to increase the number of options for decontaminating HCW in order to help make effective health security procedures. However, it is necessary to highlight that chemical decontamination processes involve health exposure, requiring knowledge regarding chemical risks and routine personal protective equipment use.

Finally, analysing the healthcare waste management process around the world is important to emphasize that when technical norms of separation have not been adhered to, all waste generated at the healthcare facility could become infected, as suggested by **Brito & Magagna (2020)**. It can increase the risk of waste management and the loss of financial resources in the process.

CONCLUSIONS

Comparing the effectiveness of the decontamination process of NaOCl, ethanol, H_2O_2, formaldehyde, and BADAC/DDAC used to disinfect concentrated broth containing bacteria *E. coli* DH5α

used in biotechnological laboratories and industries, we find a successful effect for all tested agents. Dose-response experiments demonstrated the effectiveness as described: formaldehyde > H_2O_2> NaOCl > BADAC/DDAC > ethanol. The validation of these agents on solid substrate showed that all, except BADAC/DDAC and formaldehyde, could efficiently decontaminate in a concentration of $3\times LC_{50}$. Altogether, these data suggest that NaOCl is most effective for disinfection when comparing cost, safety, and benefits; however, precaution should be taken to prevent degradation, such as wrapping the flask with foil to protect the contents from light and be alert to its short expiration date.

We highlight alternative waste treatment, *E. coli*, through the adoption of efficient, low-cost chemical products. This emphasis can be applied in the local of production (i.e., the biosafety cabinet) to inactivate the microorganism as it is produced, making it innocuous to the worker and environment. Health safety during the chemical decontamination process is also recommended to prevent associated risks, and the use of personal protection equipment is essential.

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