RESEARCH ARTICLE

In vitro inhibitory effects of commercial antiseptics and disinfectants on foodborne and environmental bacterial strains [version 1; peer review: 1 approved with reservations]

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Abstract

Background: Antibacterial agents, including disinfectants and antiseptics are commonly used to reduce bacterial loads. As they have a broad-spectrum of activity against bacteria, function either as a bactericidal or bacteriostatic. While bacterial antimicrobial resistance is increasing, disinfectants and antiseptics are still relevant antibacterial agents.

Methods: This study investigated the *in vitro* inhibitory effects of commonly used antiseptics and disinfectants. Using standard disc diffusion methods, selected common household antibacterial agents were tested on resistant *Staphylococcus aureus* isolated from hospital environment and foodborne *Escherichia coli* and *Bacillus* species.

Results: The study showed that the selected antibacterial agents were effective against the antibiotic resistant bacteria with appreciable zone of inhibition relative to the standard controls used.

Conclusions: Though bacteria are consistently developing resistance to available antibiotics, disinfectants still inhibit bacterial growth and survival with considerable public health importance.

Keywords

Antimicrobial Resistance, Antibacterial Agents, Antiseptic Antibiotics, Disinfectants, Antiseptic, Foodborne, Environmental bacteria

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Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Disinfectants and antiseptics provide a level of protection against infectious bacteria. Besides the regular use at hospitals as possible intervention against the spread of nosocomial pathogens, they also serve personal hygienic purposes and reduce bacterial loads on kitchen utensils and other home appliances, restaurants and for disinfection in research laboratories¹. While environmental and foodborne bacteria are evolving and developing resistance to available antibiotics, effective disinfectants and antiseptics inhibit bacterial growth and assured an appreciable elimination of possible contaminants². Majority of antiseptics and disinfectants are chemical agents with a broader spectrum of bactericidal and bacteriostatic activity^{1,3}. In this study, we explored the efficacy of selected randomly and regularly used antiseptics and disinfectants against selected environmental and foodborne bacterial strains in Accra, Ghana.

Methods

Bacterial strain information and antimicrobial disc preparation

Three archived bacterial strains including *Staphylococcus aureus*, *Escherichia coli* and *Bacillus* species were used in this study. These strains were obtained from AbiMosi (Abiola and Mosi) Bacterial Culture Library at West African Centre for Cell Biology of Infectious Pathogens. *Staphylococcus aureus* was isolated from hospital environmental fomites (door handles) on Mannitol Salt Agar, while *E. coli* and *Bacillus* species were isolated from food obtained from a restaurant in Ghana on MacConkey (Oxoid, England, CM0007B) and LB (Oxoid, England, CM0003) Agar at 37 °C for 24–48 h respectively. The strains were phenotypically identified with MALDI TOF MS and 16S rRNA amplification and sequencing method described by Schrottner *et al.* (2016)⁴. *Bacillus subtilis* ATCC3175 was used as the control strain. Five commercial disinfectants and antiseptics including Savlon™ (Johnson & Johnson), Camel™ (PZ cusson carex), bleach (Original Parozone – Strong and Thick Bleach 2238694, Henkel UK) CleanHome™ (Henkel, UK) and Dettol™ (Reckitt Benckiser, UK) used in this study were commercially obtained from the Accra Market in Ghana. These were selected purposively, using ease-of-access, ease-of-use, universal acceptability and relatively cheaper price. The discs were prepared using the CLSI standard for

antimicrobial-disc seeding and preparation (M02-A12). Under aseptic conditions, 25 µl of the stock and 0.1 (10⁻¹), 0.01 (10⁻²), 0.001 (10⁻³) (v/v) dilutions (in double distilled water) of the various antibacterial agents were impregnated on 6 mm wide and 0.9 mm thick (Whatman #2) filter paper sterile discs. The discs were air dried in the biosafety cabinet at room temperature for 30–45 min to allow the antibacterial agents adsorb to the discs.

Bacterial culture and antimicrobial sensitivity testing

All the media were prepared according to manufacturer's instructions and sterilized at 121°C for 15 min at 15 psi. The strains (*E. coli*, *Bacillus* spp., *S. aureus* and ATCC3175) were recovered on Luria-Bertani media (Oxoid, England, CM0003) from freezer stock. Log-phase single colonies were inoculated in sterile Mueller Hinton broth (Sigma M7408-500G; PCode: 1001876334) to obtain an optical density of 0.5 (McFarland standard, 600 nm) at 37°C for 24 h. The inoculum was seeded using sterile swab sticks on Mueller Hinton agar plates. The chemical impregnated paper discs were carefully placed, on the inoculated agar plates using sterile forceps and incubated at 37°C for 24 h, after which the zone of inhibition was measured with a meter rule. A maximum number of five (5) paper discs were placed per inoculated plate; the stock chemical, 0.1, 0.01, and 0.001 dilutions of the various antibacterial agent and cloxacillin (Mast Diagnostics, Mast Group Ltd., Merseyside, U.K.) as the positive control.

Statistical analysis

Descriptive statistics were used in this study (with SPSS 16.0 and GraphPad 6.0) and the data presented in graphs. One sample t-test and correlation analysis was done to compare the effect of the disinfectants/antiseptics on the bacterial strains using significance level of $p < 0.05$.

Results

Zones of inhibition of the five different antibacterial agents were determined using a standard grading meter rule in millimeters (Figure 1). The level of sensitivity of the bacterial strains and the efficacy of the antibacterial agents at 10⁻¹, 10⁻², 10⁻³ concentrations were determined relative to the standard antibiotic control used and ATCC3175 control (Table 1⁵).

Figure 1. Antimicrobial effect with zone of inhibitions of Savlon on a) *E. coli*; b) *Bacillus* spp. Clean Home detergent on c) ATCC3157 *Bacillus subtilis* d) *S. aureus*.

Table 1. Antimicrobial sensitivity patterns of various selected antiseptics/disinfectants.

Product	Zone of Inhibition (mm)															
	<i>E. coli</i>				<i>S. aureus</i>				<i>Bacillus</i> spp.				<i>ATCC3157</i>			
	ST	10 ⁻¹	10 ⁻²	10 ⁻³	ST	10 ⁻¹	10 ⁻²	10 ⁻³	ST	10 ⁻¹	10 ⁻²	10 ⁻³	ST	10 ⁻¹	10 ⁻²	10 ⁻³
Savlon™	23	19	14	0	NT				26	21	15	0	21	17	12	0
Camel™	26	15	10	0	23	13	10	0	20	26	15	10	18	14	11	0
Bleach™	12	13	0	0	10	10	0	0	12	12	13	0	10	10	12	0
Dettol™	26	26	0	0	15	17	0	0	NT				NT			
CleanHome™	NT				21	14	8	0	23	17	10	0	20	13	7	0
CX (5 µg)	0				0				15				0			

0 – No zone of inhibition (no antimicrobial activity), NT – (Not tested), CX – Cloxacillin (Antibiotic control), Zone of inhibition $\geq$ 10 mm indicates antimicrobial activity relative to ATCC3175 *B. subtilis* control

All strains were observed to be sensitive to the antimicrobial agents used at stock concentration.

At 0.1 and 0.01(v/v) concentrations, Savlon™, Camel™ and CleanHome™ demonstrated excellent antimicrobial activity against the tested bacterial strains. Bleach™ and Dettol™ were also effective at inhibiting bacteria growth at stock and 0.1 concentrations. Savlon™ showed highest antimicrobial activity against all the tested strains, with the smallest minimum inhibitory zone being 14 mm at 0.01 concentration, followed by Camel™ and finally CleanHome™ against *S. aureus* and *Bacillus* spp. No inhibitory activity was observed at 0.001 concentration, indicating that the antimicrobial agents lost potency with decreasing concentration (Figure 2⁵). The standard antibiotic cloxacillin (5 µg) showed effectiveness against *Bacillus* sp., but *E. coli*, *S. aureus* and ATCC3175 were resistant.

Dettol™ showed higher activity while maintaining the same zone of clearance at stock and 0.1 concentration against *E. coli* compared to Camel™ and Savlon™. At 10⁻² Savlon™ and Camel™ showed clearance as compared to Dettol™. For *Bacillus* spp. Camel™ had the highest bacterial clearance compared to CleanHome and bleach against *S. aureus* and ATCC3175. The overall average zone of inhibition of Dettol (11.75mm), Savlon (13.06mm), Clean Home (11.63mm) and Camel (12.13mm); indicating that although the strains are resistant to cloxacillin (the control standard antibiotic; CLSI 12th Edition: M02-A12), they are relatively sensitive to the antiseptics used in this study.

The analysis showed a trend of higher susceptibility of *Bacillus* spp. to the tested antimicrobials. The effects of the antimicrobials on *E. coli*, showed no difference between inhibition levels of Dettol and Savlon ($p=0.0862$) compared to Dettol and Camel ($p=0.0972$). There were significant difference between bleach and CleanHome ($p=0.0496$), Bleach and Savlon ($p=0.001$), and Savlon and CleanHome ($p=0.0389$) with *Bacillus* spp. Significant difference in inhibitory activity

between Dettol and Bleach ($p=0.0172$), Dettol and Clean Home ($p=0.0365$), and Clean Home and Camel ($p=0.0058$) with *S. aureus*. Also, it was observed that there were insignificant differences in inhibition between Bleach and Clean Home ($p=0.0631$), Bleach and Camel ($p=0.1037$), and Camel and Dettol ($p=0.0613$) (Figure 2⁵). However, for *S. aureus*, bleach had the least inhibitory activity. Overall, inhibition levels of each antimicrobial showed Savlon to be the most effective among the five antimicrobials (Mean=13.06 $\pm$ 0.01 mm), this was followed by Camel antiseptic (Mean=12.13mm $\pm$ 0.01), Dettol (Mean=11.75mm $\pm$ 0.01), Clean Home detergent (Mean=11.63mm $\pm$ 0.01), and lastly bleach (Mean=5.25mm $\pm$ 0.01).

Discussion

Foodborne and healthcare associated infections have raised growing public-health concerns in recent years^{6,7}. Apart from *S. aureus*, which is a global nosocomial pathogen and part of the normal flora, *E. coli* and species of *Bacillus* are found as residents of domestic kitchen counter tops and cutting boards; in homes, especially in bathrooms; on human skin and in the environment⁸⁻¹⁰. These bacterial strains are opportunistic and are capable of causing infections associated with the skin, blood, gut and systemic infections. Disinfection with antiseptics of potential agents of transmission, especially fomites, in homes and hospital environments has been widely recommended to reduce infection associated bacterial loads and possibly eliminate potential pathogens^{1,11} (8). A variety of commercially available disinfectants are used by the public, and from this, five commercial disinfectants (Savlon™, Camel™, Bleach, Clean Home™ and Dettol) regularly used in households and hospitals were selected for this study. These disinfectants were tested with the disc diffusion method against a variety of clinically important strains of *S. aureus*, *E. coli*, and *Bacillus* spp.

Disinfectants have broad-spectrum activity on bacteria and are usually used on abiotic surfaces as their composition

Figure 2. Efficacy of the antiseptics/disinfectants at the stock (1.000) and 0.1, 0.01, 0.001 concentrations (v/v) with $p < 0.05$.

makes them toxic to humans. In this study, Bleach was observed to have the lowest inhibitory activity against *S. aureus* and *Bacillus* spp. The composition of bleach is 3–6 % sodium hypochlorite, which is an oxidizing agent that acts by generating radicals that break DNA or RNA strands or attack the phosphate backbone of purines and pyrimidines^{12,13}. They also act on proteins and amino acids by breaking peptide bonds and break up lipids and other smaller fatty acids. These processes disrupt the normal function of bacterial cells leading to inhibition¹¹. The action of bleach is inactivated when exposed to light or heat and naturally decomposes over time. This could explain the low activity of bleach if it is in the process of decomposition or originally exposed to heat and light for long periods. Decreasing the concentration by dilution would reduce activity as the active ingredient concentration would be reduced^{13,14}.

The antiseptics used in this study composed of phenols, anilides, biguanides, and quaternary ammonium compounds². Each of the compound can be combined or used individually to achieve a desired effect. Savlon antiseptic has chlorhexidine, a bactericidal agent belonging to the biguanide family, as the main active group. It functions by damaging the outer cell layer, traversing the inner membrane and causing leakage of cell components^{2,15}. It is also known to cause coagulation of intracellular cellular components. This would explain its inhibitory effects on *E. coli* and *Bacillus* sp. Chloroxylenol is the active ingredient in Camel antiseptic; it is very effective against a wide range of gram-positive and some gram-negative bacteria^{2,14}. It disrupts cell wall due to its phenolic nature, inhibits the functions of enzymes associated with antimicrobial resistance and induces morphological changes especially in *E. coli*². Dettol contains Chloroxylenol and α -terpineol that functions like Camel and Clean Home in causing bacterial cell wall and membrane disruption especially in *E. coli* and *S. aureus*^{16,17}.

This study showed that stock antimicrobial solutions have better inhibitory activity; however, the use of stock solutions in disinfections cannot be recommended due to economic implications and toxicity. Appropriate dilutions which provide similar inhibitory activity akin to the stock solution is recommended. In the study area, cloxacillin is an effective conventional antibiotic used for treating bacterial infections. Contrary

to the expected results, cloxacillin, used as a positive control showed no significant inhibitory activity against the bacterial strains. Since bacteria resistance is sometimes rapid and spontaneous, there is a possibility that the strains have developed resistance to cloxacillin.

Conclusion

Disinfectants/antiseptics have excellent activity against potentially pathogenic bacteria circulating in homes, restaurants and hospital environments. Dettol, Savlon, Camel antiseptic, Bleach, and Clean Home detergent have antimicrobial activities. Savlon was shown to have the highest microbial inhibition in this study followed by Camel, Dettol, Clean Home, and Bleach. Dettol, Savlon, Clean Home, and Camel consistently showed appreciable levels of inhibition despite their use with different bacteria which suggests potency against a broad spectrum of microorganisms. Also, stock solutions and dilutions (concentrations not lower than 0.1) are effective in inhibiting microbial growth and therefore can be used for lab and home disinfections.

Data availability

Underlying data

Figshare: Isawumi Abiola *et al.* 2020.pdf. <https://doi.org/10.6084/m9.figshare.13060721.v1>⁵

This project contains the following underlying data:

- Isawumi Abiola *et al.* 2020.pdf (File containing raw inhibition zone measurements)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Acknowledgements

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References

1. Russell AD: **Mechanisms of antimicrobial action of antiseptics and disinfectants: an increasingly important area of investigation.** *J Antimicrob Chemother.* 2002; **49**(4): 597–599.
[PubMed Abstract](#) | [Publisher Full Text](#)
2. Maillard JY: **Antimicrobial biocides in the healthcare environment: efficacy, usage, policies, and perceived problems.** *Ther Clin Risk Manag.* 2005; **1**(4): 307–320.
[PubMed Abstract](#) | [Free Full Text](#)
3. Dettenkofer M, Wenzler S, Amthor S, *et al.*: **Does disinfection of environmental surfaces influence nosocomial infection rates? A systematic review.** *Am J Infect Control.* 2004; **32**(2): 84–89.
[PubMed Abstract](#) | [Publisher Full Text](#)
4. Schröttner P, Gunzer F, Schüppel J, *et al.*: **Identification of Rare Bacterial Pathogens by 16S rRNA Gene Sequencing and MALDI-TOF MS.** *J Vis Exp.* 2016; (113): 53176.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
5. Isawumi A, Mosi L: **Isawumi Abiola et al 2020.pdf.** *figshare.* Journal contribution. 2020.
<http://www.doi.org/10.6084/m9.figshare.13060721.v1>
6. Han JH, Sullivan N, Leas BF, *et al.*: **Cleaning Hospital Room Surfaces to Prevent Health Care-Associated Infections: A Technical Brief.** *Ann Intern Med.* 2015; **163**(8): 598–607.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Cabeça TK, Pizzolitto AC, Pizzolitto ELP: **Activity of disinfectants against foodborne pathogens in suspension and adhered to stainless steel surfaces.** *Braz J Microbiol.* 2012; **43**(3): 1112–1119.
[PubMed Abstract](#) | [Free Full Text](#)
8. Sinclair R, Gerba CP: **Microbial contamination in kitchens and bathrooms of rural Cambodian village households.** *Lett Appl Microbiol.* 2011; **52**(2): 144–149.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Becker REN, Wardenburg JB: **Staphylococcus aureus and the skin: a longstanding and complex interaction.** *Skinmed.* 2015; **13**(2): 111–9; quiz 120.
[PubMed Abstract](#)
10. Alonzo F 3rd, Torres VJ: **A lesson in survival: S. aureus versus the skin.** *Cell Host Microbe.* 2013; **13**(1): 3–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
11. Miller LG, Diep BA: **Clinical practice: colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant Staphylococcus aureus infection.** *Clin Infect Dis.* 2008; **46**(5): 752–760.
[PubMed Abstract](#) | [Publisher Full Text](#)
12. Luddin N, Ahmed HMA: **The antibacterial activity of sodium hypochlorite and chlorhexidine against Enterococcus faecalis: A review on agar diffusion and direct contact methods.** *J Conserv Dent.* 2013; **16**(1): 9–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
13. Slaughter RJ, Watts M, Vale JA, *et al.*: **The clinical toxicology of sodium hypochlorite.** *Clin Toxicol (Phila).* 2019; **57**(5): 303–311.
[PubMed Abstract](#) | [Publisher Full Text](#)
14. Hsieh SC, Teng NC, Chu CC, *et al.*: **The Antibacterial Efficacy and In Vivo Toxicity of Sodium Hypochlorite and Electrolyzed Oxidizing (EO) Water-Based Endodontic Irrigating Solutions.** *Materials (Basel).* 2020; **13**(2): 260.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
15. McDonnell G, Russell AD: **Antiseptics and disinfectants: activity, action, and resistance.** *Clin Microbiol Rev.* 1999; **12**(1): 147–179.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
16. Li L, Shi C, Yin Z, *et al.*: **Antibacterial activity of α-terpineol may induce morphostructural alterations in Escherichia coli.** *Braz J Microbiol.* 2015; **45**(4): 1409–1413.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Yoo JH: **Review of Disinfection and Sterilization - Back to the Basics.** *Infect Chemother.* 2018; **50**(2): 101–109.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Reviewer Report 12 January 2021

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Ryan G. Sinclair

Loma Linda University School of Public Health, Loma Linda, CA, USA

This is a useful assessment of bacteria that occur on fomites and the relative strength of locally available disinfectants. The authors use a ZOI method as a first pass to find the ideal type of disinfectant that can be used to disinfect 3 common target microbes. The authors chose a relatively small group of bacteria and had limited results, but have a good discussion on antibacterial agents available for the local Ghana context. I recommend indexing the paper after minor revisions.

- Please detail the type/brand of MALDI TOF and equipment used for the 16s rRNA method that you used to isolate these strains. Please detail the location of the equipment.
- Was the *Staphylococcus aureus* resistant to any antibiotics? MRSA is common in a hospital environment.
- Please detail if the 25ul of the stock solution was able to soak through the entire disc and if your team used the side of the disc that you added the solution to. I am recommending this because some liquid solutions may not soak through the entire disc and may be of a stronger concentration on one side more than the other. To address this, please add a few words to your current sentence that allows the reader to understand how well the discs accepted the different disinfectants and if they were uniformly saturated.
- Your team chose a representative group of bacteria to study from a real-world scenarios. You could expand the paper to mention that those bacteria represent a larger group of gram negative, gram positive and spore forming bacteria. The spore forming bacteria (*B. subtilis*) has also been used as a surrogate of *Cryptosporidium*.
- You should mention if your *B. subtilis* was in the vegetative state or spore form.
- A suggestion is for you to discuss the home hygiene practice that typically occurs in Ghanaian households regarding dilution. Some households typically dilute bleach in a 1:10 solution before they clean.

- It is also important to mention how well a zone of inhibition study represents a true surface contamination and disinfection study, as many microbes exist on fomites within biofilms or other substances that could lengthen their survival.
- A suggestion is that you include a sentence detailing recommendations future study. One of these is for you to do 2-fold dilutions (instead of 10-fold). Follow this up with Minimum Inhibitory Concentration (MIC) studies to find the exact concentrations required of these chemicals.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am an environmental microbiologist with experience in public health microbiology, exposure science and fomite disinfection.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 30 Jan 2021

Abiola Isawumi, Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Legon, Accra, Ghana

Excellent review and comments.

Comment: Please detail the type/brand of MALDI TOF and equipment used for the 16s rRNA method that you used to isolate these strains. Please detail the location of the equipment.

Response: The brand and version of MALDI-TOF used has been indicated under the methods used for the study (MALDI-Biotyper, Autoflex Speed, Bruker).

Comment: Was the *Staphylococcus aureus* resistant to any antibiotics? MRSA is common in a hospital environment.

Response: Yes, it was resistant to some antibiotics with regards to CLSI standard, but not included or stated in this study.

Comment: Please detail if the 25ul of the stock solution was able to soak through the entire disc and if your team used the side of the disc that you added the solution to. I am recommending this because some liquid solutions may not soak through the entire disc and may be of a stronger concentration on one side more than the other. To address this, please add a few words to your current sentence that allows the reader to understand how well the discs accepted the different disinfectants and if they were uniformly saturated.

Response: This statement has been added for clarifications "The entire disc was soaked by the stock solution and absorbed the antibacterial agents used until all the discs were uniformly saturated".

Comment: Your team chose a representative group of bacteria to study from a real-world scenarios. You could expand the paper to mention that those bacteria represent a larger group of gram negative, gram positive and spore forming bacteria. The spore forming bacteria (*B. subtilis*) has also been used as a surrogate of *Cryptosporidium*.

Response: This statement has been added "The bacterial strains selected for this study represent the most common Gram-positive and Gram-negative bacteria. Also, *B. subtilis* has been used as a surrogate of *Cryptosporidium*, a microscopic parasite that causes diarrheal diseases".

Comment: You should mention if your *B. subtilis* was in the vegetative state or spore form.

Response: The *Bacillus* species tested was at the sporulation state.

Comment: A suggestion is for you to discuss the home hygiene practice that typically occurs in Ghanaian households regarding dilution. Some households typically dilute bleach in a 1:10 solution before they clean.

Response: It is difficult to provide information on this, as no survey was done prior to the study. The study has already suggested that stock solution of majority of the disinfectants is effective and recommended, 1:10 for some as observed in the study.

Comment: It is also important to mention how well a zone of inhibition study represents a true surface contamination and disinfection study, as many microbes exist on fomites within biofilms or other substances that could lengthen their survival.

Response: The ZOI compared to standard antibiotics as used in this study was guided by CLSI protocols and have been used to evaluate effectiveness of antibacterial agents in relation to disinfection of surfaces in the lab, hospitals, etc. The information provided in the study suits its justification.

Comment: A suggestion is that you include a sentence detailing recommendations future study. One of these is for you to do 2-fold dilutions (instead of 10-fold). Follow this up with

Minimum Inhibitory Concentration (MIC) studies to find the exact concentrations required of these chemicals.

Response: This suggestion has been adopted under the 'conclusion section'.

Competing Interests: No competing interest