In vitro inhibitory effects of commercial antiseptics and disinfectants on foodborne and environmental bacterial strains [version 2; peer review: 1 approved, 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 bactericidal or bacteriostatic agents. 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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Author roles: Isawumi A: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; Donkor JK: Conceptualization, Investigation, Methodology, Visualization; Mosi L: Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Introduction
Disinfectants and antiseptics provide a level of protection against infectious bacteria. Aside from their regular use at hospitals as possible intervention against the spread of nosocomial pathogens, they also serve personal hygiene purposes and reduce bacterial loads on kitchen utensils and other home appliances, restaurants and for disinfection in research laboratories. 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 contaminants. Majority of antiseptics and disinfectants are chemical agents with a broader spectrum of bactericidal and bacteriostatic activity. 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 at sporulation were used in this study. 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. 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 (MALDI-Biotyper, Autoflex speed, Bruker) and 16S rRNA amplification and sequencing method described by Schrottner et al. (2016). *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 2283694, Henkel UK) CleanHome™ (Henkel, UK) and DettoF™ (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 entire disc was soaked by the stock solution and absorbed the antibacterial agents used until all the discs used were uniformly saturated. 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 *ATTCC3175*) 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 ATTC3175 control (Table 1). All strains were observed to be sensitive to the antiseptics 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 2). The standard antibiotic cloxacillin (5 µg) showed effectiveness against *Bacillus* sp., but *E. coli*, *S. aureus* and ATTC3175 were resistant.

Detto™ 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 Detto™. For *Bacillus* spp. Camel™ had the highest bacterial clearance compared to cleanHome and bleach against *S.aureus* and ATTC3175. The overall average zone of inhibition of Detto (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 Detto and Savlon ($p= 0.0862$) compared to Detto and Camel ($p= 0.0972$). There were significant difference between bleach and cleanHome ($p= 0.0496$), Bleach

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Zone of Inhibition (mm)</th>
<th>Zone of Inhibition (mm)</th>
<th>Zone of Inhibition (mm)</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E. coli$</td>
<td>$S. aureus$</td>
<td><em>Bacillus</em> spp.</td>
<td>ATCC3157</td>
</tr>
<tr>
<td></td>
<td>$10^{-1}$</td>
<td>$10^{-2}$</td>
<td>$10^{-3}$</td>
<td>$10^{-1}$</td>
</tr>
<tr>
<td>Savlon™</td>
<td>23</td>
<td>19</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Camel™</td>
<td>26</td>
<td>15</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Bleach™</td>
<td>12</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dettol™</td>
<td>26</td>
<td>26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CleanHome™</td>
<td>NT</td>
<td>21</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>CX (5 µg)</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
</tbody>
</table>

0 – No zone of inhibition (no antimicrobial activity), NT – (Not tested), CX – Cloxacillin (Antibiotic control), Zone of inhibition ≥ 10 mm indicates antimicrobial activity relative to ATCC3157 *B. subtilis* control.
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 2). 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 ±0.01 mm), this was followed by Camel antiseptic (Mean=12.13mm±0.01), Dettol (Mean=11.75mm±0.01), Clean Home detergent (Mean=11.63mm±0.01), and lastly bleach (Mean=5.25mm±0.01).

**Discussion**

Foodborne and healthcare associated infections have raised growing public-health concerns in recent years. 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 tops and cutting boards; in homes, especially in bathrooms; on human skin and in the environment. 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. 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 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. 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 inhibition. 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.

The antiseptics used in this study composed of phenols, anilides, biguanides, and quaternary ammonium compounds. 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 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. 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. 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.

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 different bacteria which suggests potency against a broad spectrum of microorganisms.

**Data availability**

**Underlying data**


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**

The authors would like to thank West African Centre for Cell Biology of Infectious Pathogens (WACCBIP) and Department of Biochemistry, Cell and Molecular Biology for providing facilities for this study. Mosi Lab for technical supports, Edwin Kyei-Baffour for report collation and the MCBI-MPhil Class of 2021 students that conducted this research as a part of the MCBI 603 Experimental Microbiology course between August and October 2019; thanks for your diligence and commitment to research excellence.

**References**

Open Peer Review

Current Peer Review Status: ✔️ ❓

Version 2

Reviewer Report 22 July 2021

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Inmaculada García-Romero

Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

The article is well written and describes an interesting topic. In this pandemic time, we all are concerned about the efficiency of the disinfectants we use at home to kill viruses and bacteria. The authors analyzed a set of disinfectant products acquired from the Accra Market in Ghana which are commonly used in houses and clinics, using a small set of bacterial strains to measure the ZOI at different concentrations of the disinfectants. I believe it is an interesting piece of work to publish in this journal; however, I have some comments:

Mayor comments:

1. How many replicates were done for the ZOI? The standard it is at least three biological replicates. If the authors did just one, they should justify why or complete the replicates and calculate the SD of them, including the error bars in the Figure 2 and the SD in the values of Table 1 and, finally, use the data of all the replicates to perform the statistical analysis.

2. Why did the authors not check all the disinfectants for all the strains? They need to justify why or test them.

Minors comments:

1. In the introduction, I missed some references to other papers in which the authors did similar work. Here there is an example: Wanja et al. (2020).

2. In the methods, the authors mention that they did 16s rRNA amplification. The sequence of the oligonucleotides used for the amplification should be included.

3. Sometimes the authors write 10⁻¹, 10⁻² and others 0.1, 0.01 to indicate the dilution of the disinfectants. This should be uniform.

4. In the Figure 1, the dilution factors are not clearly visible in all the plates.

5. In the footnote of the Table 1, the authors say; “...Zone of inhibition ≥ 10 mm indicates...”
antimicrobial activity relative to ATCC... control”. Why? Could the author explain this sentence? How do they normalize the values with the control? This should be included in methods.

6. In Figure 2, the number of zeros in the x-axis values should be uniform.

7. At the end of the conclusions: “...that higher dilution factor,...” I think the authors mean lower dilution factor. Check that sentence.

References

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? Partly

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

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

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

Are the conclusions drawn adequately supported by the results? Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Antibiotic resistance, microbiology, molecular microbiology, bioinformatic.

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.
Ryan G. Sinclair
Loma Linda University School of Public Health, Loma Linda, CA, USA

I have no further comments to make. The authors considered my comments and updated their manuscript.

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?
Yes

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

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

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.
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