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The Effect of Varying Chemicals upon Antibacterial Effectiveness Against *E. coli* Growth

Abstract

Research Question: What is the antibacterial effectiveness of diverse chemicals, incorporating store-bought, commercially produced antiseptics and traditional homemade remedies, against *E. coli* growth, as indicated by the zone of inhibition?

In order to address the research question, an experimental approach was utilised. Samples of *E. coli* bacteria were spread evenly upon agar plates, subsequent to which paper discs, soaked in a range of chemical substances, were deposited to designated sections on the agar plates. Following incubation, and thus culturing of the *E. coli*, the zone of inhibition, representing the area where bacterial growth was successfully inhibited, was measured. An analysis of the results revealed that whilst commercially produced antiseptics exhibited potent antibacterial properties, traditional homemade antiseptics displayed varying degrees, typically limited, of inhibitory effects upon *E. coli* growth. The findings of this study may assist in directing individuals to selecting appropriate, maximally effective disinfection methods, which is particularly topical in today's post-pandemic era.

Introduction

Bacteria may be generally identified as prokaryotic organisms, that, thereby, lack membrane-bound organelles, are unicellular, possess a single circular chromosome within the nucleoid region of the cytoplasm (refer to Figure 1), and undergo cell division by binary fission (Clark and Pazdernik, 2013). Moreover, bacterial cells are categorised into four principal types, according to their configuration: the *Staphylococcus, Bacillus, Spirillum,* or *Vibrio* groups, which respectively label spherical, rod-shaped, spiral, or curved bacterial structures (Pappas and Vidyasagar, 2021).

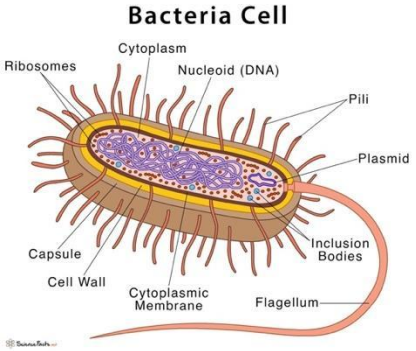

Figure 1: A diagram depicting the cellular structure of a bacterial cell, which, furthermore, appears to belong to the *Bacillus* group owing to its rod-shaped, cylindrical structure. (Mukherjee, 2020).

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Escherichia coli (E. coli) may be defined as a rod-shaped, and thus *Bacillus*, Gram-negative bacterium, that is a foodborne pathogen (Lim, Yoon, and Hovde, 2010) which typically populates the lower intestinal tract of warm-blooded animals, and is therefore capable of discharge by means of, for instance, faeces, (Jang et al., 2017). The vast majority of *E. coli* types are lacking of the potential to instigate any severe harm – the extent to which illness may be caused includes diarrhea, urinary tract or bloodstream infections, and respiratory illness (CDC, 2023). Accordingly, the minimally adverse consequences of *E. coli* have led to its commonplace use in laboratory settings; for example, as will be utilised in the following experiment, the K-12 *E. coli* strain is a debilitated, laboratory-derived sample, which is non-pathogenic and frequently commercially exploited (Perna et al., 2002). Such *E. coli* may be cultured in vitro – and exponential growth will thus occur – for diverse research, industrial, and diagnostic objectives, by means of inoculating a small number of *E. coli* cells into a sterile medium volume (Elbing and Brent, 2018). Said *E. coli* growth, or overall bacterial culturing, may be inhibited and killed by bactericidal agents, which are often in liquid solution form (Pankey and Sabath, 2004).

Two conventional types of bactericidal solution include disinfectants and antiseptics; the former which are chemicals operated to target bacteria colonies on non-living, inanimate surfaces, whilst the latter kills microbe growth upon skin and mucous membranes to prevent sepsis (Brennan, 2021). Disinfectants typically function through three predominant mechanisms of action – cross-linking, coagulating, clumping; structure and function disruption; and oxidising (Thompson, 2012), whilst antiseptics generally penetrate the lipid bilayer of the cell membrane, or inhibit overall cellular processes through, for instance, denaturing proteins (Savlon, n.d.). Both substances are considered ineffective against bacterial spores (Rayasam et al., 2023).

The zone of inhibition (refer to Figure 2), resultant of a Kirby-Bauer test (LibreTexts, 2018) may be defined as the circular area, visible subsequent to incubation, enfolding a particular location at which a bactericidal agent is situated, distinguishing the region within which bacterial colonies do not grow (Bhargav et al., 2016).

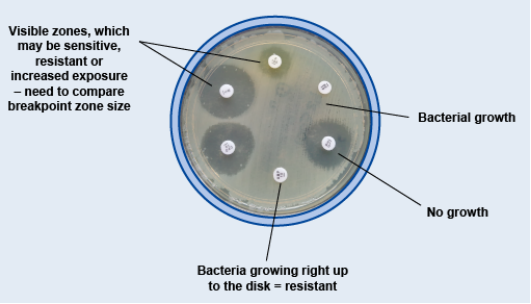

Figure 2: A labelled image of different zones of inhibition for different antibiotic disks, subsequent to incubation of the agar plate. (The Fleming Fund, 2021).

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Its measure by means of, for example, the length of the circle from a consistent point, or the diameter or radius, may act as a qualitative measure of the susceptibility of a bacterial species against a specific bactericidal agent, thereby determining the effectiveness of the said agent (Bayston, 2017). Namely, the standard relationship between the two factors is that a larger zone of inhibition indicates a greater level of sensitivity of the bacterium to the bactericidal agent, suggesting reasonable effectiveness of the agent substance (BiologyWise, 2015).

Hypothesis

If the bactericidal solution is commercially produced, then it will be more effectual in deterring bacterial growth, and thereby, the zone of inhibition will be averagely larger, in comparison to homemade solutions.

> **Reasoning:** The hypothesis assumes commercially produced bactericidal solutions are produced by reputable professional companies, and therefore undergo rigorous testing and standardisation that is regulated by industry protocols, in comparison to homemade solutions which do not have inhibiting bacterial growth as their original purpose.

Variables

Independent variable: Type of bactericidal solution (Dettol, Betadine, sodium chloride solution, vinegar, water).

Dependent variable: Diameter of the zone of inhibition (mm).

Controlled variables	Reasons for being kept constant
Brand of agar and size of the agar plates.	Maintaining the agar brand establishes a standardised baseline allowing fair comparison, given varying brands may contrast in, for example, composition and performance quality. Moreover, it ensures reproducibility, through culturing consistently proportional colonies of bacteria. The size of the agar plates must be controlled, as an increase in surface area may correlate to increased bacterial growth.
The E. coli strain (K-12), and the volume of E. coli.	Keeping the E. coli strain constant, as K-12, ensures certain bacterial characteristics remain constant, such as growth rates or virulence factors, which contributes to a fair test. Controlling the volume of E. coli live broth is significant for consistent bacterial inoculation, permitting the impact of the independent variable to be accurately comparable.
The volume of chemical substances within which the paper discs were soaked.	The concentration of chemical substance on the paper discs remaining consistent, allows a more accurate assessment on the substance's effect on E. coli growth, as potential

	variability is reduced due to standard diameter of diffusion into the agar.

Table 1: *Controlled variables and their impact.*

Table 2: *Uncontrollable variables and their potential impact upon experimental data.*

Uncontrollable variables	**Potential impact on data**
Genetic variability of the E. coli within the K-12 strain.	Genetic variability in the form of spontaneous mutations may cause phenotypic differences, varying antibiotic resistances, introducing variability and hence degrading the quality of precision in the experimental data.
Paper disc absorption rates.	Minute differences in thickness, density, and size, of the paper discs, influence absorption rates. This may lead to slightly uneven diffusion of substances from the paper discs into the agar mediums may cause inaccurate bacterial growth patterns, downgrading the accuracy of the data.
Contamination of agar plates due to factors such as airborne microorganisms.	Despite only opening the agar plates at a 45-degree angle to best prevent contamination, due to the populous nature of airborne microorganisms, it is uncontrollable some may contaminate the agar plates. Fair testing may be impacted negatively, as it is unknown if the data results may be wholly contributed to the independent variable.

Apparatus

- 5 × sterile agar (refer to Appendix C) plates
- 10mL *Escherichia coli (E. coli)*, K-12 strain, live broth
- 1 × inoculating loop
- 1 × glass spreader
- Hole punch
- Filter paper
- Tweezers
- Forceps
- 5 × 50mL beakers
- 15mL Betadine antiseptic liquid
- Sodium chloride (NaCl) solution (1%) 1.0g/L
- 250mL Dettol classic antiseptic liquid
- 375mL Cornwell's white vinegar

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- Tap water
- Sticky tape
- Adhesive labels
- Marker
- Plastic tray
- Incubator (30ºC)

Method

Section 1.0 Preparation of the Agar Plates

1. Utilising a permanent marker, divide the bottom of the five sterile agar plates, in their closed state, into four equal sections each (refer to Appendix A).
2. Label all four sections of one agar plate as '1'.
3. Label one of the four sections on the remaining four agar plates each, respectively as 'a', 'b', 'c', and 'd'.
4. Place five drops of the *E. coli* live broth onto the centre of four agar plates, excluding the plate labelled with '1', ensuring the plate lids are only opened to 45º whilst doing so with the inoculating loop.
5. Sterilise the glass spreader through heating it in the flame of the Bunsen Burner.
6. Gently swathe the entire agar surface by spreading the bacteria culture with the spreader.

Section 1.1 Preparation of the Paper Discs

7. With the hole punch, create 16 paper discs from the filter paper.
8. Fill a 50mL beaker with 10mL of tap water.
9. Repeat Step 6, replacing tap water with 1% sodium chloride (NaCl) solution, Dettol classic antiseptic liquid, and Cornwell's white vinegar.
10. Label the beaker containing water as '1', the beaker containing Dettol as 'a', vinegar as 'b', NaCl as 'c', and Betadine as 'd' (refer to Appendix B).
11. Using tweezers, deposit four paper discs each into the five 50mL beakers, and ensure they are sufficiently soaked in the respective chemical solutions.

Section 1.2 Assembly of the Experimental Agar Plates

12. Lightly press each of the paper discs to stick them to the agar surface, placing them in the centre of a section. (refer to Appendix A) – correspond the label of the beaker within which the disc was soaked and the label of the agar plate section. For instance, place the paper discs soaked in Dettol in the agar plate sections labelled 'a'.
13. Place the lid on the agar plate, and firmly sticky tape the entire side of the lid to the base of the agar plate.
14. Label the agar plates with initials and the date of assembly.

Section 1.3 Incubation of the Agar Plates

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15. Turn the agar plates upside down, and place them in the incubator.
16. Incubate at 30 ℃ for 48 hours.

Method Justification

A qualitative experimental method was selected to determine the susceptibility of *E. coli* growth against certain bactericidal solutions, including Dettol, Betadine, sodium chloride solution, and vinegar – this variability ensures two commercially produced antiseptics and two common homemade antiseptics were included respectively, allowing direct comparison of their resulting data. The method incorporates a control group, ensuring fair test, represented by an agar plate labelled '1' and treated with normal tap water. This serves as a baseline standard to allow evaluation of experimental conditions, and the effects of the other chemical treatments. Moreover, the method specifies the use of five sterile agar plates, comprising the control group, indicating each treatment will have four replicates and thus, the sample size is reasonable, as it includes an extent of replication.

Safety and Ethical Risks

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☐ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: Emma Choi ID:

SCHOOL: Loreto College Marryatville

Activity: Give a brief outline of what you are planning to do.

This experiment intends to test the antibacterial effectiveness of diverse chemicals, comparing the effects of commercially produced, store-bought antiseptics and traditional homemade remedies, against E. coli growth, as indicated by the zone of inhibition. Filter paper discs soaked in varying antiseptic solutions will be placed on agar plates, and bacterial growth will be promoted in an incubator at 30°C for 48 hours. Resultingly, the size of the zone of inhibition will indicate an antiseptic's effectiveness and capacity to inhibit and kill E. coli growth.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could

Table 3: *Possible hazard risks and ethical considerations, and the measures taken to maximise their prevention.*

Risk	**Control/Management**
Spillage: Due to the presence of various liquid solutions, include sodium chloride solution and tap water, in the necessary experiment materials, spillage of these could result in a slipping hazard and induce injury.	Ensuring methods that concerned the handling of fluids were carried out over a work bench surface, and handling liquids with general caution, will assist in mitigating this hazard. Upon the occurrence of a spillage, an absorbent material may be utilised to mop it up, and potential injuries may be tended to with first aid.
Glassware: The utilisation of certain apparatus, such as 50mL measuring beakers, that are prone to breakage and may potentially shatter into fragments upon shock contact, could instigate physical wounds and lacerations.	Delicate and gentle usage in order to reduce the prospective of apparatus damage, as well as the donning of appropriate safety equipment, such as glasses, will assist in alleviating this risk. Upon the sustaining of an injury, it may be disinfected by antibacterial wipes and bandaged securely.
Chemicals: Potentially improper storage and use of chemical antiseptics, such as Betadine and Dettol, could have, upon exposure to skin and eyes, led to allergic conjunctivitis and dermal hypersensitivity reactions.	Warranting the solutions were in good condition prior to the experiment must be given precedence to, through, for example, ensuring their date of expiration has not been passed. If required, an irritated area of an allergic reaction may be treated by first-aid procedures.
Bacteria: Despite the harmlessness of agar itself, the bacteria – in this case, *E. coli* – cultured, has the potential to be pathogenic and therefore cause disease or illness upon exposure.	The agar plates will be tightly sealed with sticky tape subsequent to the required method being carried out and prior to incubation, and they will not be re-opened for the remainder of the experiment.
Electrical: Given an incubator will be utilised to promote bacterial growth on the agar plates, possible contamination and electrical issues with cord damage are possible.	The cord of the incubator will be inspected thoroughly prior to conducting the experiment for any forms of damage or heat corrosion – if any impairment is noticeable, the cord will be replaced immediately.

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Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)): Emma Choi

SIGNATURE(S): Emma Choi

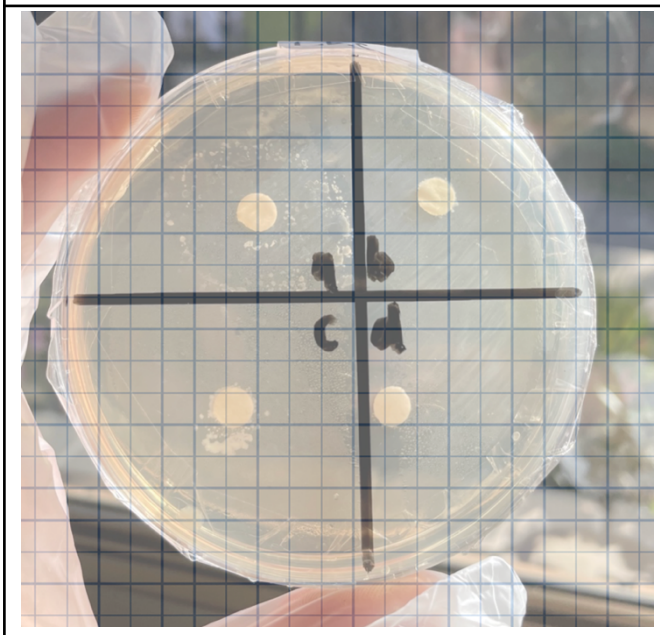
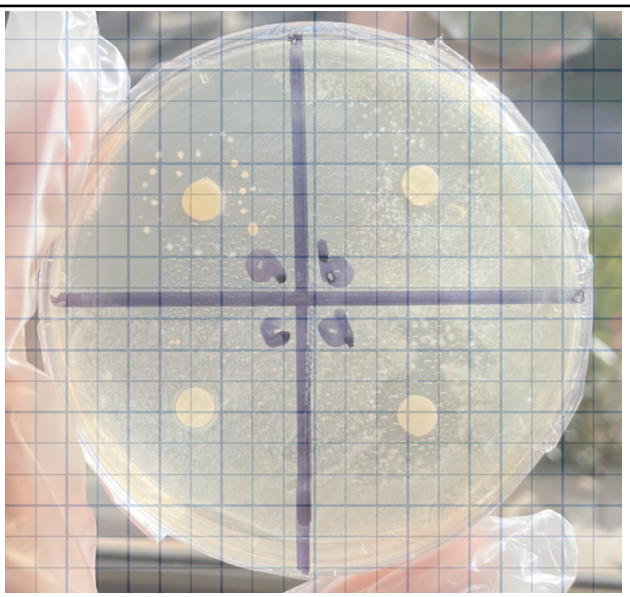
☐ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: Mrs Patty Warrender

SIGNATURE: Warrender DATE: 09/05/2023

Results

Table 4: *Photographs of the agar plates subsequent to incubation, overlayed by a 5mm × 5mm grid (refer to Appendix D).*

Trial 1	**Trial 2**
	
Trial 3	**Control group**

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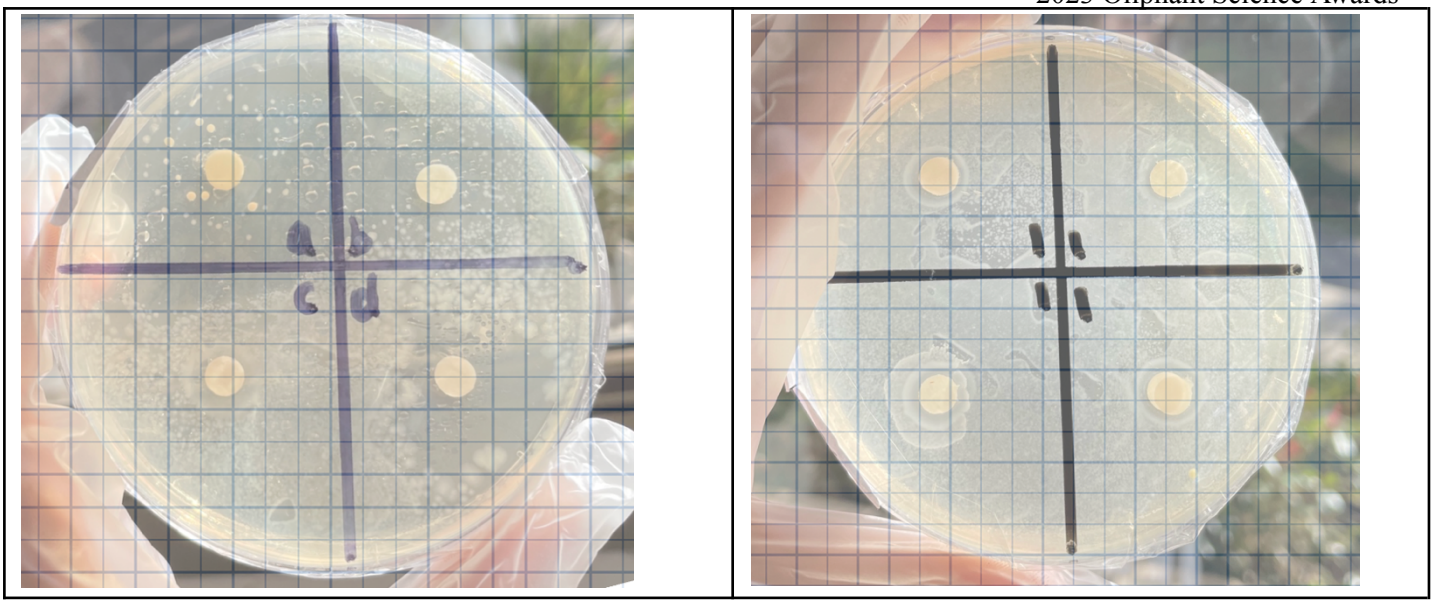

General observations:

- The photo quality was somewhat negatively impacted due to the slight disruption of clarity by the inclusion of the gloved hand, particularly in the control group image
- The largest zone of inhibition, overall, appears to be contributed to ‘d’ (Betadine) in Trial 3
- The zones of inhibition are not precise, perfect circles – instead, they are irregularly shaped, indicating a consistent point of measurement will be necessary, and that the radius nor the diameter per say may not be determined
- Certain E. coli colonies seem stippled, such as in ‘a’ (Dettol) of Trials 2 and 3, whilst others possess a ‘cloudy’ appearance, such as in ‘c’ (sodium chloride solution) of Trials 1, 2, and 3
- An outlier may have occurred in ‘d’ (Betadine) of Trial 1, given its zone of inhibition is minimal in area compared to its equivalent bactericidal solution in Trials 2 and 3

Table 5: *The effect of the type of bactericidal solution upon the zone of inhibition (mm) (the zone of inhibition was measured from the lowermost point of the paper disc to the bottom edge of the circle).*

	Zone of Inhibition (mm)			
Type of Bactericidal Solution	**Trial 1**	**Trial 2**	**Trial 3**	**Average**
Dettol	9	6	6	7
Betadine	9	6	13	9
Sodium chloride solution	0	0	0	0
Vinegar	0	3	5	3
Water	0	0	0	0

Table 6: *The standard deviation zone of inhibition values for Trials 1, 2, and 3 of the respective bactericidal solutions.*

Type of Bactericidal Solution	**Standard Deviation of the Zone of Inhibition (mm)**

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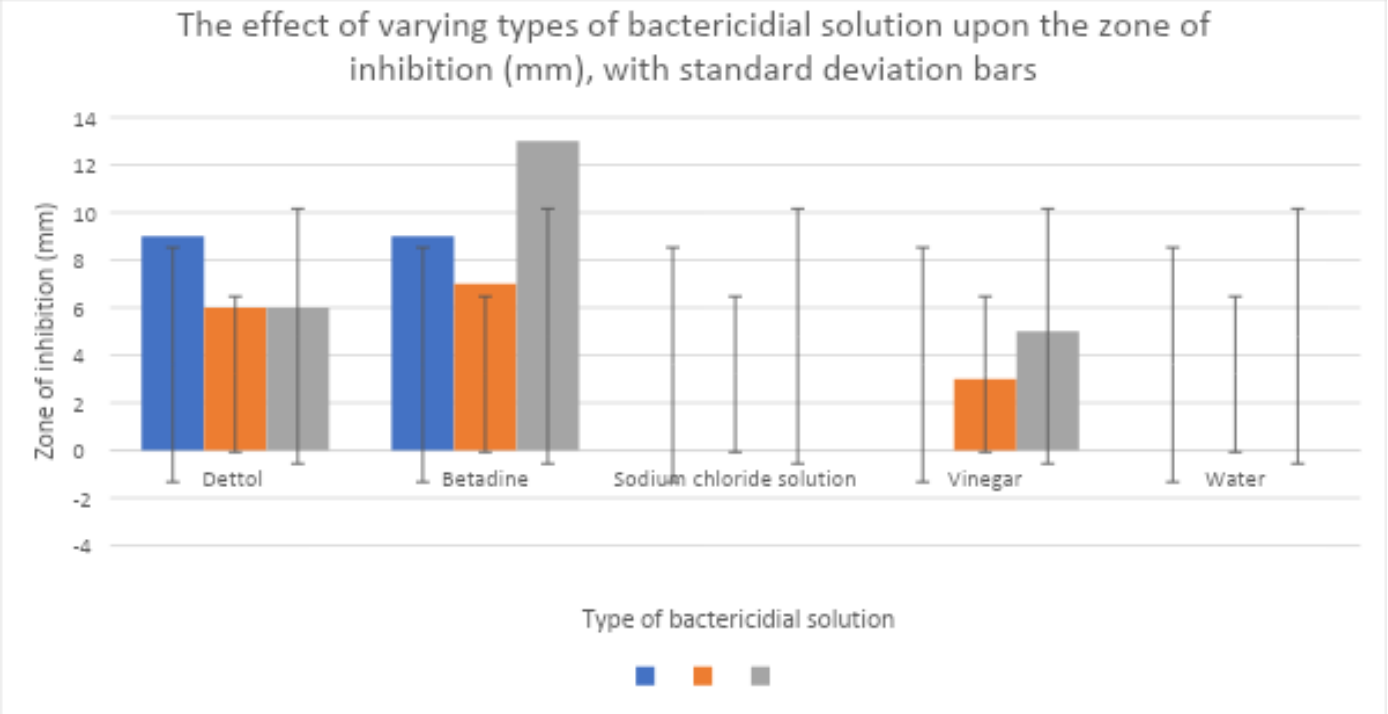
Dettol	1.414
Betadine	2.867
Sodium chloride solution	0.000
Vinegar	2.055
Water	0.000

Figure 3:



Discussion

Discussion of Results

Table 5 exhibits an increase in the average size of the zone of inhibition generally corresponding to a commercially produced bactericidal solution, in comparison to a homemade remedy; thereby, the hypothesis statement was supported. As evaluable from Figure 3, Dettol and Betadine antiseptic liquids exhibited moderate inhibitory effects upon *E. coli* bacterial growth – Betadine, in Trial 3, demonstrated a moderate to strong inhibitory effect, with there being a substantial zone of inhibition increase from the consistent 6mm sourced in Trials 1 and 2, to 13mm. This divulges limited precision, and the potentially more operational bactericidal properties of Betadine in comparison to Dettol. In contrast, Figure 3 additionally demonstrates the lack of any zone of inhibition formations throughout all trials of sodium chloride solution and water, the latter of which was expected, as the control group. Furthermore, vinegar, although being classified a homemade bactericidal solution, still displayed a relatively low inhibitory effect,

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with an average zone of inhibition of 3mm. Thereby, the bactericidal solutions may be ordered in the following order of the most effective to the least – Betadine, Dettol, vinegar, sodium chloride, and water.

The results are accurate, and parallel applicable scientific knowledge. For example, the commercial antiseptic solutions of Betadine and Dettol contain specialised, active ingredients, that have antimicrobial properties as their primary function. Consequently, Betadine includes povidone-iodine (refer to Appendix E), which is capable of killing a range of bacteria, viruses, and fungi, through lipid iodination and oxidation of cytoplasmic and membrane compounds (NIH, 2011), whilst Dettol contains chloroxylenol (refer to Appendix F), an antimicrobial which exhibits bactericidal properties through mechanisms including cell membrane disruption, protein denaturation, and interference with enzymatic operations (PubChem, n.d.). Provided povidone-iodine is largely considered more potent than chloroxylenol (Majidipour, Abdeyazdan, Zargham-Boroujeni, 2013), the experimental results, displaying Betadine to be averagely more effective at preventing *E. coli* growth than Dettol, may be concluded to be true. Furthermore, water was successfully utilised as the control group substance, as it is a universal solvent ineffective against hydrophobic bacteria which often simply displaces bacteria (Riza and Syaflida, 2018). Likewise, sodium chloride solution was ineffective against bacterial growth as it usually simply creates a more alkaline environment wherein bacteria do not thrive (Leiva, 2021) – given, however, agar is an optimal environment for bacterial growth, this was not applicable. However, white vinegar was capable of slight bacterial inhibition, due to its containment of acetic acid and particular efficacy against *E. coli* as a species (Nunez, 2020).

Nonetheless, certain data inconsistencies imply accuracy is still flawed, to an extent; for example, in Table 5, the zone of inhibition being measured at 9mm in Trial 1 and 6mm in Trial 2, of testing the effect of Betadine, is distinctively inconsistent with the finding that Betadine is the most successful inhibitor of E. coli growth, which is otherwise supported by the mean experimental data. This is owing to a 9mm zone of inhibition being consequential of Trial 1 of Dettol, and 6mm zones of inhibition additionally being recorded in Trials 2 and 3. Hence, systematic errors likely contributed towards the relegation of data accuracy.

General Evaluation

A potential systematic error is the incorrect calibration of the incubator, and therefore possible temperature disparities or the presence of a temperature gradient. This may have resulted in differing temperatures in sections of the incubator, leading to skewed variations in bacterial growth due to the inconsistency of heat provision to the agar

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plates. Secondly, improper sterilisation of the glass spreader or inoculating loop may have led to contamination, distorting the accuracy of the results.

As evident in Table 5, the presence of standard deviation values – particularly for Betadine, with approximately 2.867 – demonstrate low precision, with extensive data scatter, which would have been instigated by random error. It is perceivable in Figure 3 that whilst the standard deviation bars possess substantial length, depicting overall poor precision, Trial 2 had the least overall scatter, and thus may be deemed most reliable of the three trials. Random errors include human error in the timing of the exact 48 hours for which the agar plates must be incubated, inconsistent levels of contamination of the agar with airborne microorganisms during the period for which they are open for paper disc placement, and minutely different volumes of *E. coli* broth provided to each of the agar plates due to the vagueness of the restriction '5 drops'.

Finally, certain aspects of the experimental design, such as four trials having been carried out every time the independent variable was altered, and the presence of a control group, enhance the validity. However, improvements in the experiment, such as sealing the petri dishes with a material, like parafilm, that is more reliable to prevent contamination than sticky tape, and utilising distilled water instead of tap water to prevent introducing contaminants, may augment the accuracy.

Conclusion

The findings demonstrated that of the five bactericidal solutions tested, Betadine, Dettol, vinegar, then sodium chloride solution and water, respectively, were most efficacious in inhibiting *E. coli* growth – this upholds the hypothesis, given Betadine and Dettol are commercially produced and the remaining substances are typically homemade. Whilst accuracy was reasonable, precision was somewhat poor as conveyed by the extensive data scatter; diverse errors reduced data quality, and warranting a fair test in the experimental design positively impacted validity.

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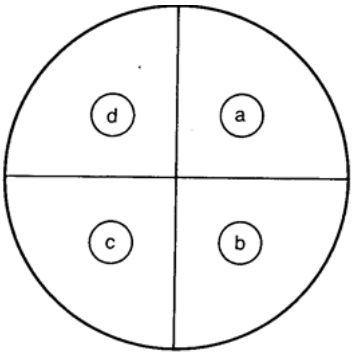
The Fleming Fund (2021) *OLCreate: Fleming Fund F Antimicrobial susceptibility testing: 3.3 Measuring and interpreting the test*. Available at: https://www.open.edu/openlearncreate/mod/oucontent/view.php?id=172394&section=4.3 (Accessed: 27 June 2023).
[Figure 2]

Thompson, K. (2012) *The Science Of Disinfectants*, *Cleaning & Maintenance Management*. Available at: https://cmmonline.com/articles/the-science-of-disinfectants (Accessed: 27 June 2023).

Appendices

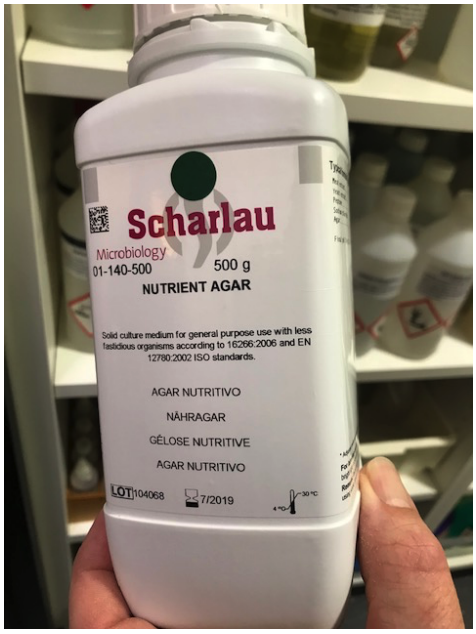
Appendix A: A prepared agar plate, depicting the four drawn divided sections, with four respective paper discs in position with different chemicals.

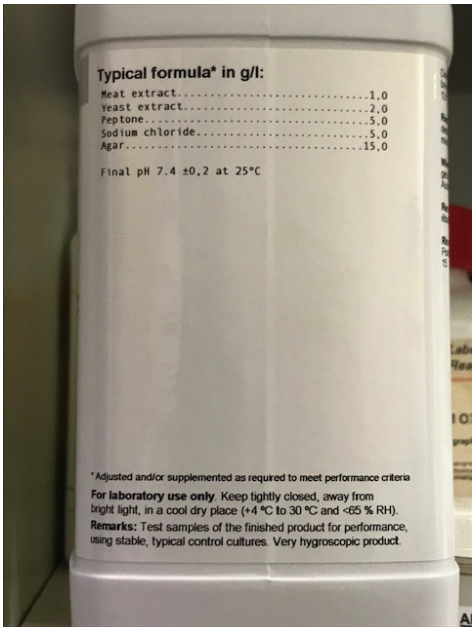


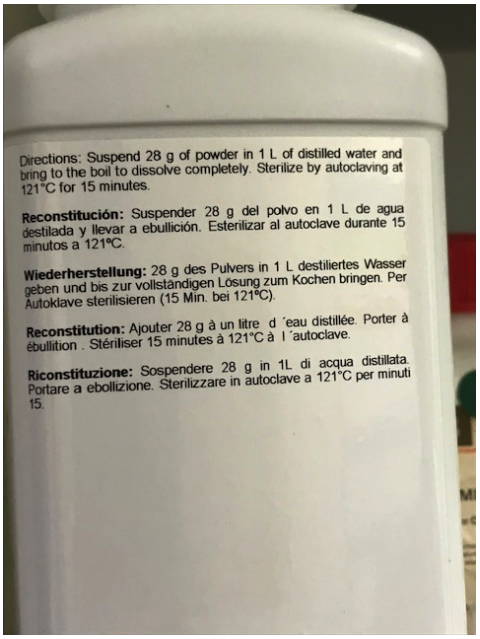
Appendix B: A photo of the five labelled 50mL beakers, containing the five different chemical antiseptic solutions.

Word Count: 2176 (excluding h , and logbook)

Appendix C: Ingredients of the agar utilised, as well as the recipe utilised.

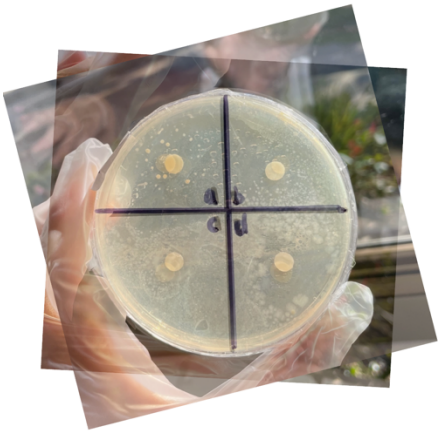




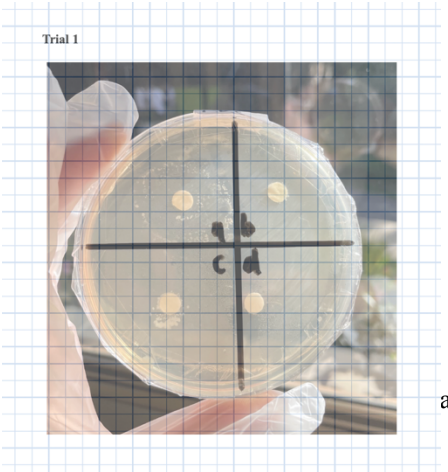


Appendix D: Digitally measuring the zone of inhibition**.**

Firstly, utilising each of the images that were taken of the agar plates, they were aligned via an image transparency tool, ensuring that the petri dishes were of the same approximate size and the markings were lined up accordingly, as such:



Afterwards, an accurately scaled A4 grid, with measurements of 5mm x 5mm per grid square, was overlayed:



Word Count: 2176 (excluding headings, appendices, and logbook)

Appendix E: The ingredients of Betadine antiseptic liquid.

Appendix F: The ingredients of Dettol classic antiseptic liquid.

Experimental Research Logbook

04/04/2023 Tuesday

I began formulating potential experimental research questions for investigation, that were all essentially rooted in my interest in medical science and healthcare:

1. How does UV light exposure impact the viability of varying skin cell types?
2. What is the effect of fluctuating antibiotic concentrations upon bacterial growth?
3. How do varying pH levels influence the rate of catalytic enzyme activity?

Word Count: 2176 (excluding headings, titles, figure captions, tables, references, appendices, and logbook)

4. How do different air pollutants and their concentrations affect respiratory function?
5. What is the impact of temperature upon the movement of aspirin across a semi-permeable membrane?

05/04/2023 Wednesday

I deconstructed Question 1, through the following brief research and conclusions:

- Ultraviolet light (UV) light may be defined as a type of electromagnetic radiation, originating from the sun and transmitted in waves or particles at varying wavelengths (the range of which is defined the electromagnetic (EM) spectrum) and frequencies
- UV radiation is classified as a 'complete carcinogen', given it acts as a mutagen capable of both tumour initiating and promoting; thereby, it is amongst one of the most prominent risk factors for skin cancer
- Therefore, skin cells are a relevant cause of investigation; specific types may include keratinocytes, melanocytes, and fibroblasts, which may be exposed to UV light sources of varying intensities in a potential experimental design
- However, due to concerns of biological considerations with respect to sourcing skin cells, and hazards of dealing with intense levels of UV light, this experimental possibility was placed low on the preference list

Sources

Lucas, J. (2017) *What Is Ultraviolet Light?*, *livescience.com*. Available at: https://www.livescience.com/50326-what-is-ultraviolet-light.html (Accessed: 5 April 2023).

D'Orazio, J. *et al.* (2013) 'UV Radiation and the Skin', *International Journal of Molecular Sciences*, 14(6), pp. 12222–12248. Available at: https://doi.org/10.3390/ijms140612222.

06/04/2023 Thursday

Question 2 was deconstructed with the following:

- Antibiotics are essentially drug-form medications that inhibit bacterial growth, and are prescribed in order to treat bacterial infections
- Bacterial responses to antibiotics are concentration-dependent – at high concentrations, antibiotics exhibit antimicrobial activities upon susceptible cells, whereas subinhibitory concentrations induce diverse biological responses in bacteria
- If selected for a laboratory experiment, it must be ensured an agar medium lacks any antibiotics, creating a control group, and that the same type of antibiotic should be maintained throughout the entire experiment, with simply concentrations differing

Sources

Felman, A. and Bagum, F. (2019) *Antibiotics: How they work, uses, side effects and how to use*. Available at: https://www.medicalnewstoday.com/articles/10278 (Accessed: 6 April 2023).

Word Count: 2176 (excluding headings, titles, figure captions, tables, references, appendices, and logbook)

Bernier, S.P. and Surette, M.G. (2013) 'Concentration-dependent activity of antibiotics in natural environments', *Frontiers in Microbiology*, 4, p. 20. Available at: https://doi.org/10.3389/fmicb.2013.00020.

07/04/2023 Friday

Question 3 was deconstructed with this information:

- Enzymes are biological catalysts, and changes in their structure, particularly tertiary, influence enzymatic activity
- For instance, in terms of pH, the optimal pH value will caused maximally efficient enzymatic activity, and under the value, reaction rate will be on an increasing incline; however, beyond the optimum pH, enzymatic activity decreases and plateaus
- As I wanted to focus on a more medical-focused research question, and given I had already completed a similar experiment for a school assignment, I completely eliminated this from my options

Sources

Effect of pH on Enzymatic Reaction - Creative Enzymes (no date). Available at: https://www.creative-enzymes.com/resource/effect-of-ph-on-enzymatic-reaction_51.html (Accessed: 30 June 2023).

08/04/2023 Saturday

Question 4 was researched and deconstructed, as following:

- Especially with the modern environmental crisis, the health effects of air pollution, including particulate matter or nitrogen dioxide, are a significant public worldwide health concern – it is capable of inducing acute exacerbation of COPD and onset of asthma, for example
- However, potential limitations of designing an laboratory experiment in correspondence with this research question is difficulty in gathering results, as it is difficult to measure 'respiratory function'
- The possible need for a sample population of organisms, such as human subjects or animal models, would raise major ethical concerns – thus, this research question was also eliminated from further investigation

Sources

Jiang, X.-Q., Mei, X.-D. and Feng, D. (2016) 'Air pollution and chronic airway diseases: what should people know and do?', *Journal of Thoracic Disease*, 8(1), pp. E31–E40. Available at: https://doi.org/10.3978/j.issn.2072-1439.2015.11.50.

09/04/2023 Sunday

Question 5 was researched and deconstructed:

- Aspirin (acetylsalicylic acid) is a pharmaceutical drug utilised to reduce symptoms of pain and inflammation, recently associated with the prevention of blood clot formation

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- I consulted the school laboratory technician regarding the possibility of utilising water baths to alter the temperature, and investigating its impact upon the movement of aspirin through a semi-permeable membrane, modelling this said membrane with dialysis tubing

Sources

Asprin - Alcohol and Drug Foundation (no date). Available at: https://adf.org.au/drug-facts/aspirin/ (Accessed: 9 April 2023).

10/04/2023 Monday

Question 2 was given preference to, and therefore experimental design and further refinements were closely considered whilst researching further:

- Due to being inspired by discussion of antiseptics and disinfectants in class, I altered Question 5 to focus on different types of antiseptics, rather than different antibiotic concentrations; I found this especially relevant in today's post-pandemic era
- Therefore, various characteristics of antiseptic substances that could be compared in effectiveness were considered – for instance, expensiveness of the product
- Ultimately, I decided to compare commercially produced and homemade antiseptics – this was prompted by discussion with a teacher regarding their past experiences with disinfecting lacerations with vinegar, and I felt this encapsulated the cost-effectiveness aspect as well
- Given that I was testing solution substances, I decided employing filter paper, in the form of hole-punched discs, would be more effective than any form of swabbing, which is often a common technique
- Following discussions with various classmates and teachers, I narrowed down four selections of different antiseptics, two commercially produced and two homemade remedies, to test – Dettol, Betadine, salt water, and vinegar were the most common, and thus were selected; water was added as a control group

11/04/2023 Tuesday

Subsequent to comprehensive investigation, of the five potential research questions, an extension of Question 2 was selected and refined to create the final inquiry: What is the antibacterial effectiveness of diverse chemicals, incorporating store-bought, commercially produced antiseptics and traditional homemade remedies, against *E. coli* growth, as indicated by the zone of inhibition? In turn, I conversed with my science teacher, Mrs Patty Warrender, regarding a possible trial attempt at utilising an agar plate for bacterial growth, and a date for this was confirmed on Wednesday this week.

12/04/2023 Wednesday

During class time, I swabbed a microphone in the music room of the school, and a door handle to the general science lab. Following swabbing, I traced an 'S' shape on the surface of the agar in an agar plate, and handed it up, subsequent to sealing and labelling, for incubation by the school laboratory technician. This allowed me to both become familiar

with the utilisation of agar plates, such as learning that they are placed in the incubator upside down due to condensation, and practice safety when dealing with bacterial colonies, such as only ever opening the agar plate lid at a 45º angle and never opening a sealed agar plate following incubation.

14/04/2023 Friday

The agar plates from Wednesday were provided for observation, as they had undergone incubation and thus had colonies of bacterial growth.

The experimental design was developed and refined specifically throughout the period between Monday to Friday.

09/05/2023 Tuesday

A risk assessment was put together with reference to the planned experimental design, and the Oliphant Science Awards risk assessment form was signed and scanned.

12/05/2023 Friday

The practical experiment was carried out today, and the agar plates were successfully treated; they were set aside on a plastic tray for the school laboratory technician to incubate over the weekend, for the intended 48 hours.

15/05/2023 Monday

Ensuing incubation over the Saturday and Sunday, the agar plates were collected, and photos were taken in order to successfully digitally measure the zone of inhibition (refer to Appendix D). For the purpose of close observation, general comments regarding the incubated agar plates, regarding for instance, their appearance, were noted, to include in the Results section of the practical report.

Word Count: 2176 (excluding headings, titles, figure captions, tables, references, appendices, and logbook)