Extended Essay

Biology

Investigating different bactericidal effects of disinfectants and traditional methods on Staphylococcus Aureus and Escherichia Coli

Research question:

Do disinfectants containing chemicals (active chlorine; ethyl alcohol, isopropyl alcohol; active chlorine released from sodium hypochlorite, hydrogen peroxide) and non-chemical traditional methods (lemon juice, wine vinegar, and niaouli oil) have same bactericidal effects on Staphylococcus aureus and Escherichia Coli as indicated by clear zone diameter formed in the blood-agar plate with the antibiogram test?

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Introduction

The idea of investigating different bactericidal effects of disinfectants and traditional methods came into my mind because of the COVID-19 which started to spread and caused a pandemic in late 2019. Over 6 million people lost their lives and over 600 million cases occurred due to this virus ***(Worldometer, 2024)***. To increase and to minimize the transmission rate of the virus and to protect society from the virus, many precautions have implemented worldwide like social distancing, wearing masks, contact tracing and hand sanitization. The awareness of hand sanitization has highly raised during the pandemic and, although they were also used before the pandemic, during this period they became a very important part of our lives and we started to keep them in our bags no matter what which made more disinfectant brands to occur then before.

Besides the usage of the disinfectants against bacteria and viruses, the existence of some traditional methods and usage of them has also highly decreased the bacteria rates. Although these traditional methods had an important place as external agents over ages, I have examined that the disinfectants are used more often in the society because of their practical usage. I wondered whether the substances of the disinfectants, caused by containing different chemicals, and different traditional methods have different bactericidal effects on bacteria.

Research Question

The specific research question answered in this investigation is: Do disinfectants containing chemicals (active chlorine; ethyl alcohol, isopropyl alcohol; active chlorine released from sodium hypochlorite, hydrogen peroxide) and non-chemical traditional methods (lemon juice, wine vinegar, and niaouli oil) have same bactericidal effects on Staphylococcus aureus and Escherichia Coli as indicated by clear zone diameter formed in the blood-agar plate with the antibiogram test.

Background Information

1. Bacteria

Both bacteria and viruses can be spread easily from one person to another via hands, which are mostly used for contacting with another person that facilitates the spread of the bacteria. Because hands have various areas of use from simple actions to complex activities, they create an easy and common route for the spread of infectious diseases. When someone contacts with objects or surfaces that others usually contact with their hands, they encounter with the mucous membranes of body which can be infectious ***(Drexler, 1970)***. In this investigation, it is chosen to take bacteria samples from human hands where moisture levels influence bacterial growth positively besides the jammed environment of them.

As illustrated above, there are so many bacteria that can live on human hand since it has an appropriate environment for bacterial growth. Staphylococcus Aureus and Escherichia Coli are the most common bacteria which can grow on human hand easily so that Staphylococcus Aureus and Escherichia Coli are chosen as bacteria samples in this experiment. E. Coli is a gram-negative bacterium so that, as well as having probability of having a double-layered and wavy peptidoglycan layer, it has a probability of having a single-layered peptidoglycan. Illnesses which are caused by this bacterium are usually non-harmful, but they can also cause body ailments or gastrointestinal infections ***(Mueller, 2023)***. On the other hand, Staphylococcus Aureus is another type of bacteria that can cause various infections in humans. Having a single-layered and smooth cell wall and a thick peptidoglycan layer that can be also multi-layered, S. Aureus is a gram-positive bacterium. This bacterium does not cause serious infections unless it is allowed into bloodstream or internal tissues ***(Taylor, 2023)***. These two bacteria are preferred in this investigation because they have a cloudy appearance on the agar plate so they can be seen easily with naked eye making it easy to collect data and to investigate. Besides the feature of their appearance, they are less harmful than another bacterium.

2. Foreign Agents

To prevent the multiplication of bacteria, foreign agents like disinfectants are used. As mentioned before, during the Pandemia in 2020, the usage rate of the disinfectants is highly increased. Even though this precaution of disinfectants were mostly for the Corona Virus, it

helped with the prevention of bacterial spread. Disinfectants are foreign agents, and they kill or inhibit the growth of bacteria by destroying the protective membrane which covers the bacteria. At the time the membrane is destroyed, the internal structure and contents of the bacteria will leak out and functions of them will be prevented too ***(Bell-Young, 2023)***. In hospitals and for daily usage, there are three different types of common disinfectants that contain active chlorine; ethyl alcohol, isopropyl alcohol; active chlorine released from sodium hypochlorite, hydrogen peroxide. In this investigation these 3 types of disinfectants will be used.

2.1. Disinfectants

The first disinfectant mainly contains active chlorine which is a powerful against viruses or bacteria and commonly used as a disinfectant. Active chlorine disturbs the structure and the function of biological molecules in microorganisms while rapidly effecting the mycoplasma and vegetative bacteria with the absence of an organic load ***(HICPAC, 2016)***. Ethyl alcohol (*ethanol*) and isopropyl alcohol (*isopropanol*) are included in the second disinfectant. Besides these alcohols can be used as disinfectants in household or healthcare, they are also used as cleaning products in daily life. Ethyl alcohol is a powerful disinfection agent which can inactivate wide range of pathogens when used at concentrations of 60% to 80% as well as Isopropyl alcohol. When these two alcohols are combined, their disinfecting abilities highly enhance ***(HICPAC, 2016)***. In the last disinfectant, Sodium hypochlorite (*NaClO*) and hydrogen peroxide (H_2O_2) were possessed. When sodium hypochlorite dissolves in water, it releases active chlorine in the form of hypochlorous acid (*HOCl*) and the hypochlorite ion (OCl^-) and, these forms are responsible for disinfection of sodium hypochlorite ***(National Library of Medicine,2014)***. Hydrogen peroxide, which is a chemical compound that contains two hydrogen atoms and two oxygen atoms, releases oxygen when it meets organic materials. With this ability, it can destroy microorganisms. When hydrogen peroxide and sodium hypochlorite are combined, the oxidation potential of the solution increases so that the disinfectant's effectiveness increases, and it gets easier to break down organic material ***(National Library of Medicine,2014)***. Alternatively, Bleach or absolute alcohol could be used in this investigation. However, they can be extremely drying and irritating to the skin. So, they are not used for daily disinfection for skin. Because of

this, daily used disinfectants are preferred, which are not harmful and do not have toxic effects for skin health.

2.2. Traditional Methods

While modern scientific approaches and medical interventions are critical, traditional methods rooted in cultural practices and common sense can also contribute to public health efforts. Instead of chemical containing disinfectants some people preferred using non-chemical traditional methods. Three different traditional methods are used in this experiment: lemon juice, wine vinegar, and niaouli oil. The first traditional method is lemon juice has an acidic nature and it has presence of compounds like citric acid. These properties of lemon juice make it act as an antibacterial agent. Lemon juice is highly acidic that has a low pH level so that it has an unstable condition for bacterial life. Citric acid disrupts the cell membrane of bacteria, interfering with their metabolic processes and potentially causing them to die or become less active ***(ER Ekawati and W Darmanto, 2019)***. The second used traditional method is wine vinegar which has antibacterial properties because of its acidic nature. The acetic acid in vinegar creates an acidic environment that can inhibit the growth and survival of certain bacteria. When high concentration of solutes in vinegar contact with bacterial cells, they create an osmotic imbalance which draw water out of bacterial cells and cause them to lose their structural wholeness. While vinegar makes bacterial cells shrink, it leads to bacterial cell death ***(Angela Betsaida B. Laguipo, 2019)***. The last used traditional method is niaouli oil which is an essential oil derived from the leaves of the Niaouli tree. This oil contains active compounds like cineole, alpha-pinene, and terpinen-4-ol. With the existence these compounds, niaouli oil possesses antibacterial properties against a range of bacteria so that it can inhibit the growth and proliferation of bacteria ***(National Library of Medicine, 2021)***. Instead of investigating the bactericidal effects of disinfectants and traditional methods, some sterilization processes' (boiling water, steaming) bactericidal effects can be investigated too but these sterilization processes are not suitable for hand disinfection.

3. Agar Plates and Antibiogram Test

Even though the main target of this investigation is the bacteria on human hand, it won't be possible to do an experiment on a living being. Instead of using real human hand as experiments place, samples from hands are taken. So that blood agar plates used which contain nutrients from red blood cells providing essential growth factors for the bacterial cells ***(Libretexts, 2020)***.

After the investigation, a data must be collected to achieve results. To obtain this, antibiogram test is used in this investigation for collecting data. Antibiograms are valuable methods which rapidly provide a summary of the resistance of pathogenic microorganisms to specific agents' bactericidal effects ***(National Library of Medicine, 2021)***. Using antibiogram and measuring the clear zone on it is chosen because antibiogram tests ensures the accurate results in a short time zone in about 1 to 2 days.

Hypothesis

It is predicted that disinfectants containing chemicals (active chlorine; ethyl alcohol, isopropyl alcohol; active chlorine released from sodium hypochlorite, hydrogen peroxide) and non-chemical traditional methods (lemon juice, wine vinegar, and niaouli oil) don't have same bactericidal effects on Staphylococcus aureus and E. Coli's clear zone diameter formed in the blood-agar plate with the antibiogram test.

Hence, clear zone formed around the antibiogram papers of disinfectants will be larger than traditional methods. Disinfectants will be more effective on preventing bacterial life of Staphylococcus aureus and Escherichia Coli bacterium.

Method

1. Variables

Independent Variable: Type of the cleansing agent

Whether the substances of the disinfectants, caused by containing different chemicals, and different traditional methods have different bactericidal effects on bacteria will be the subject to this investigation. The type of the cleansing agent will be changed using traditional methods other than disinfectants.

Dependent Variable: Diameter (mm) of the clear zone of the antibiogram on the blood agar plate containing bacteria.

Since bacterium life will be distributed because of the used cleaning agents, this will reflect throughout the diameter (mm) of the clean zone around the antibiogram papers which are soaked into cleaning agents. While the type of cleansing agents will be changing, the clean zone diameters will differ from each other too.

Controlled Variables:

The volume of cleansing agent	Using different volume of cleaning agent for each sample will result with not being significant. While the clear zone amount of a sample using more cleaning agent may be larger, the clear zone may be less in a different sample because of using less cleaning agent. By using the same amount of disinfectant and traditional methods, the differences in the effects of these cleaning agents can be observed.
Temperature	After bacterial samples are collected, a certain temperature is needed for them to grow and multiply. An incubator is used to achieve this. Therefore, the environment should be controlled, and incubator should be used at 36 degrees centigrade. If the temperature of the incubator is used differently, the growth or development of bacteria won't be possible.
Type of agar medium	In order for bacteria to grow and multiply, an agar plate that is close to the human hand should be used. The agar plate closest to this is the blood agar plate. If a different agar plate is used for each bacterial sample in the experiment, bacterial growth and proliferation will vary.
Type of bacteria used	The growth rate and proliferation time of each bacterium may vary. If bacteria from different species are used in the experiment, the experiment will not be significant.

Table1: Controlled Variables; amount of cleansing agent, temperature, type of agar plates, type of bacteria used

2. Safety, Environment and Ethical

Because bacteria have been used in this investigation, it is important to take strong precautions. For safety, bacteria mostly less harmful for the human body and the bacteria that are normally found in the human body, mostly in hands, were selected. The used bacteria do not cause serious infections unless they enter bloodstream or internal tissues. It is very important to use medical gloves and medical face masks in this experiment to prevent these bacteria from entering the bloodstream. For the ethics of the environment, chemical waste garbage and recycling garbage used in laboratories should be used for the waste occurred after the investigation. To prevent serious diseases, help was received from professional laboratory staff of Koru Private Hospital.

3. Apparatus and Materials

*Medical gloves

*Medical face mask

*6 medical cotton swabs

*6 blood agar plates

*Inoculating loop

*Micro sterilizer

*Incubator (36 degrees centigrade)

*10 mg of disinfectant containing active chlorine

*10 mg of disinfectant containing ethyl alcohol and isopropyl alcohol

*10 mg of disinfectant containing active chlorine released from sodium hypochlorite and hydrogen peroxide

*10 mg of lemon juice

*10 mg of wine vinegar

*10 mg of niaouli oil

*30 antibiogram papers

*Millimetric ruler

4. Procedure

-Put on medical gloves and medical face mask.

Growing and spreading bacteria

1) Take a bacteria sample from a specific person's uncleaned fingers and palm with the medical cotton swap.

2) Contact the used medical cotton swap with the blood agar plate and scatter around it.

3) Take an inoculating loop and put it into the micro sterilizer for 3 seconds to purify the inoculating loop.

4) Slightly spread out the bacteria sample, which has put previously, ensuring that it contacts everywhere of the blood agar plate with the clean inoculating loop.

5) Close the blood agar plate with its cover.

6) Repeat the first 5 steps on different blood agar plates for 5 more time.

7) Put the 6 blood agar plates with the bacteria samples into incubator which has a temperature of 36 degrees centigrade.

8) Wait for 48 hours for the fully growth and spread of the bacteria.

Preparation of antibiogram papers

9) Soak 5 antibiogram papers with 10 mg of disinfectant containing active chlorine.

10) Soak 5 antibiogram papers with 10 mg of disinfectant containing ethyl alcohol and isopropyl alcohol.

11) Soak 5 antibiogram papers with 10 mg of disinfectant containing active chlorine released from sodium hypochlorite and hydrogen peroxide.

12) Soak 5 antibiogram papers with 10 mg of lemon juice.

13) Soak 5 antibiogram papers with 10 mg of wine vinegar.

14) Soak 5 antibiogram papers with 10 mg of niaouli oil.

15) Make sure that all the antibiogram papers have absorbed the agents fully.

Antibiogram tests

16)Remove the agar plates from the incubator after 48 hours and label each agar medium with a name of each cleaning agent in a circular form.

17) For each blood agar plate, which has bacteria grown on it, place 5 antibiogram paper containing respective disinfectants written on its label, which absorbed the same type of cleansing agent, with an equal distance.

18) Repeat the 16^{th} step for 6 blood agar plate. Place antibiogram papers on blood agar plates marked with the agents which each antibiogram paper absorbed.

19) Put the 6 blood agar plate into the incubator and wait for the results for 24 hours.

20) Take out the 6 blood agar plate after 24 hours.

21) Measure the diameter of clear zones occurred around each 30 antibiogram paper using a millimetric ruler.

Data Collection

1. *Raw Data*

Type of cleansing agents	Cleansing agents	Trial number	Clear zone (mm±0.5)
Traditional Methods	Lemon juice	1	10.0
		2	5.0
		3	4.0
		4	7.0
		5	1.0
	Niaouli oil	1	13.0
		2	8.0
		3	6.0
		4	2.0
		5	7.0
	Wine vinegar	1	9.0
		2	5.0
		3	4.0
		4	6.0
		5	5.0
Disinfectants	Active chlorine	1	4.0
		2	0.0
		3	5.0
		4	0.0
		5	4.0
	Ethyl alcohol-Isopropyl alcohol	1	2.0
		2	3.0
		3	3.0
		4	0.0
		5	0.0
	Active chlorine released from sodium hypochlorite-Hydrogen peroxide	1	5.0
		2	2.0
		3	10.0
		4	3.0
		5	4.0

Table 2: Raw Data Table; clear zone (mm) based on the type of cleaning agent for each cleaning agent

2. *Processed Data*

	Average clear zone diameter(mm)	StdDev	StdError
Active Chlorine	**2.6**	**2.4**	**1.1**
Ethyl Alcohol, Isopropyl Alcohol	**1.6**	**1.5**	**0.7**
Active Chlorine Released from Sodium Hypochlorite, Hydrogen Peroxide	**4.8**	**3.1**	**1.4**
Lemon Juice	**5.4**	**3.4**	**1.5**
Niaouli Oil	**7.2**	**4.0**	**1.8**
Vinegar	**5.8**	**1.9**	**0.9**

Table 3: Average clear zone diameter (mm), standard deviation, standard error

Average clean zones of each data group are calculated with the formula of: $\mu = \frac{\sum X}{N}$

$$\mu_{Lemon\ Juice} = \frac{10 + 5 + 4 + 7 + 1}{5} = 5.4$$

Standard deviations of the agent groups are calculated with the formula of: $\sigma = \sqrt{\frac{\sum(X-\mu)^2}{N}}$

For Lemon Juice;

Count, N: 5

Sum, Σx: 27

Mean, $\bar{x}$: 5.4

Variance, s^2: 11.3

$$\sigma_{Lemon\ Juice=}\sqrt{\frac{(10-5.4)^2 + \cdots + (1-5.4)^2}{5-1}} = 3.3615472627943 \approx 3.4$$

Standard errors of each data group are calculated with the formula of: $SE = \frac{\sigma}{\sqrt{n}}$

$$SE_{Lemon\ Juice} = \frac{3.3615472627943}{\sqrt{5}} = 1.5033296378 = 1.5$$

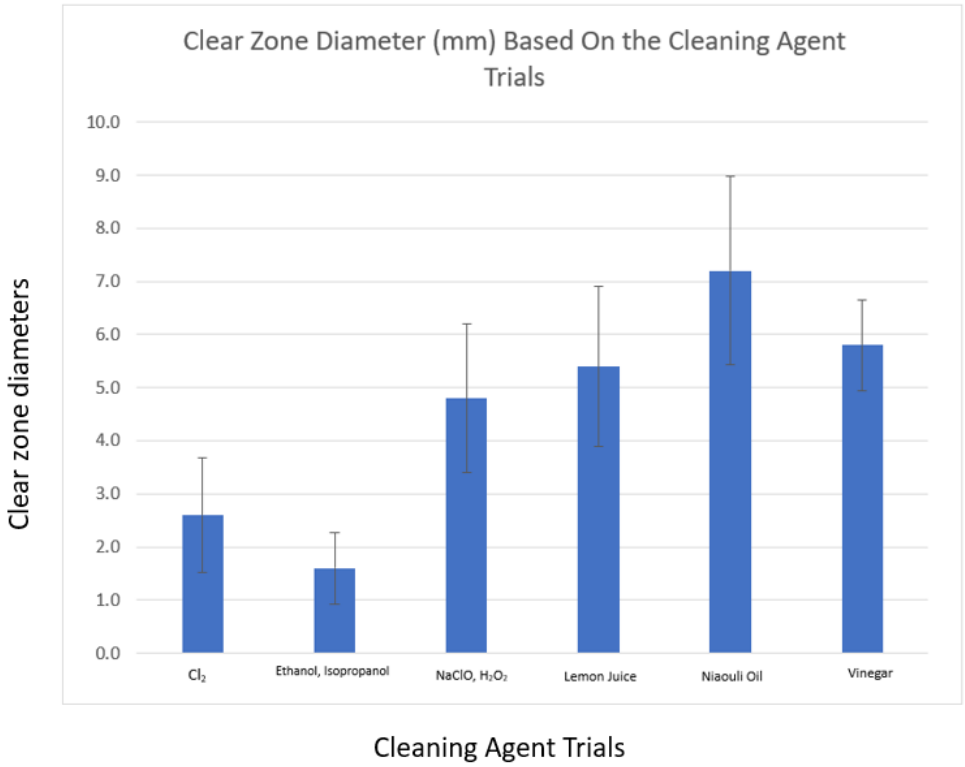
3. *Hypothesis- H_A and H_0*

The null hypothesis of the investigation is: There is no statistically significant difference between average clear zone diameters among different cleaning agents. The difference can be explained by chance.

$$H_0: \mu_{L.J.} = \mu_{V.} = \mu_{N.Oil} = \mu_{A.Chlorine} = \mu_{Ethyl\ A.,Isopropyl\ A.} = \mu_{A.C.Released\ from\ S.Hyp.,Hydrogen\ P.}$$

The alternative hypothesis of this investigation is: There is a statistically significant difference between average clean zone diameters among different cleaning agents. The difference is too large to be explained by chance.

$$H_A: \mu_{L.J.} \neq \mu_{V.} \neq \mu_{N.Oil} \neq \mu_{A.Chlorine} \neq \mu_{Ethyl\ A.,Isopropyl\ A.} \neq \mu_{A.C.Released\ from\ S.Hyp.,Hydrogen\ P.}$$



Graph 1: Average clear zone diameter(mm) for each cleaning agent trials, error bars drawn using StdError values

4. *ANOVA Results*

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between the Groups	**109.3667**	**5**	**21.87333**	**2.705979**	**0.044685**	**2.620654**
Within the Groups	**194**	**24**	**8.083333**			

Table 4: ANOVA, conducted using excel

5. *Analysis of The Results*

When the raw data table is examined, it is seen that the traditional methods have more bactericidal effects compared to disinfectants. When the processed data table **(Table 3)** is taken into consideration, Active Chlorine; Ethyl Alcohol, Isopropyl Alcohol; Active Chlorine Released from Sodium Hypochlorite; Hydrogen Peroxide; Lemon Juice; Niaouli Oil; Vinegar have mean clear zone diameter values of 2.6, 1.6, 4.8, 5.4, 7.2, 5.8 millimeters respectively. As seen in data, the traditional methods have more bactericidal effects than disinfectants. So that disinfectants containing chemicals and non-chemical traditional methods have different bactericidal effects on Staphylococcus Aureus and E. coli's clear zone diameter formed in the blood-agar plate with the antibiogram test. As seen in **Graph 1**, the most effective traditional method is Niaouli Oil then Vinegar and the least effective traditional method is Lemon Juice. The most effective disinfectant is Active Chlorine Released from Sodium Hypochlorite, Hydrogen Peroxide, then Active Chlorine and the least effective disinfectant is Ethyl Alcohol, Isopropyl Alcohol. With these values, the three traditional methods have higher average clean zone diameters than disinfectants proving that traditional methods are more effective in killing Staphylococcus aureus and Escherichia Coli bacteria compared to disinfectants.

Even though there is difference among mean values of all cleaning agents and there is a minor overlap in the error bars, which were provided using Standard Error values **(Graph 1)**, it can be understood that the differences between the mean values did not come about by chance when the results of ANOVA are taken into consideration. The p-value of the data is 0.044685 as seen in

Table 4. Because the p-value of the data is smaller than the value of $\alpha = 0.05$, it can be understood that the data results are statistically significant. Null hypothesis H_0 can be rejected with the result of p-value. Because there is a statistically significant difference between cleansing agents and their bactericidal effects Alternative Hypothesis can be accepted.

$$p\ value = 0.044685 < 0.05$$

With this p-value, the results cannot occur by chance.

The hypothesis of the investigation is particularly true which was: It is predicted that disinfectants containing chemicals (active chlorine; ethyl alcohol, isopropyl alcohol; active chlorine released from sodium hypochlorite, hydrogen peroxide) and non-chemical traditional methods (lemon juice, wine vinegar, and niaouli oil) don't have same bactericidal effects on Staphylococcus aureus and Escherichia Coli's clear zone diameter formed in the blood-agar plate with the antibiogram test. Even though the disinfectants and traditional methods do not have same effects against Staphylococcus aureus and Escherichia Coli, it was predicted that the disinfectants will be more effective than traditional methods. However, the data results show that the traditional methods are more effective than disinfectants.

Conclusion

The specific research question that the investigation aimed to answer was: Do disinfectants containing chemicals (active chlorine; ethyl alcohol, isopropyl alcohol; active chlorine released from sodium hypochlorite, hydrogen peroxide) and non-chemical traditional methods (lemon juice, wine vinegar, and niaouli oil) have same bactericidal effects on Staphylococcus aureus and Escherichia Coli as indicated by clear zone diameter formed in the blood-agar plate with the antibiogram test? The calculations of clear zone diameter were made on Blood Agar Plate Cultures of bacteria prepared by spread plate technique.

Disinfectants were expected to be more effective, hence, clear zone diameters around antigram papers soaked in disinfectants were expected to be larger. The hypothesis was based on presence of chemicals in the disinfectants. Disinfectants have an ability of rapidly effecting the mycoplasma while effecting a wide range of bacteria. Although traditional methods had a major

place as cleaning agents over ages, it was examined that the disinfectants are used more often in the society because of their practical usage.

However, the result of the investigation shows that the traditional methods have stronger bactericidal effects. Since the impact range of disinfectants is wide, their effect on bacteria could increase over a larger range. The experiment was performed on blood agar medium, and this might have decreased the effectiveness. Another reason for the result obtained is that disinfectants act quickly. While the blood agar plates were waiting in the incubator, the effectiveness of the disinfectants may have expired, and bacteria may have started to reproduce on the clean zone. Since the effective time of traditional methods weren't short, the clear zone diameter at the end of the period was larger. Thus, it can be said that traditional methods have more bactericidal effects than disinfectants in the long term.

Evaluation

1. Strengths

   The amount of data collected is one of the strengths of this investigation. By conducting 5 trials of 6 different cleaning agents, each with different contents, generalization of results was achieved.

   The average clean zone diameters all show that the results are significant. All traditional method trials have a larger value of mean then the disinfectants that proves the result's significance.

   Due to antibiogram test made on the results were achieved fast and easy. This calculating method was not expensive when it is compared to other calculation methods like spectrophotometric analysis or viability assessment method. Another advantage of this method is antibiogram papers and blood agar plates were easily accessible, thus making it easier to carry out the experiment.

2. Weaknesses and Improvements

The diameter of clean zone around the antibiogram papers were collected with a millimetric ruler. With this way of collecting data, minor errors could be made. (mm∓0.5) The data collection of diameters of clear zone could be made using cumulative antibiogram report and analyzing it so this technology would ensure the results which could be more precise and significant. This report method could not be used because it was not available in the laboratory worked in. Another weakness is the wide range of bacteria on human hand. Since not all bacteria on the hand can be detected, another type of bacteria other than Staphylococcus aureus and Escherichia Coli may have affected the experiment. Although these bacteria might have a minor effect, biochemical tests could also be performed in addition to identifying the bacteria under the microscope.

3. Further Investigation

In this investigation, the bacteria samples were collected from human hand. Even though hands crate a suitable environment for bacteria multiplication and growth, some surfaces, where bunch of individuals contact daily, crate a suitable environment for bacterial spread. Instead of getting samples from hands, samples could be taken from surfaces like elevator buttons, door handles or phones. The kind of bacteria will vary if it was taken from the surfaces so that the used cleaning agents. In another bacteria-focused experiment, appropriate disinfectants could be used to clean surfaces and the effects of these disinfectants could be observed.

Our elders use surface cleaner cleaning agent for house cleaning. These cleaning agents encounter human skin during cleaning and sometimes they cause damage to the hands. Since these cleaning agents contain chemicals suitable for surface cleaning, they could have different effects on people. As another experiment, the effects of these cleaning agents on humans could be examined. Thus, an experiment focusing on the cleaning agent was created and a result regarding biological safety could be obtained.

Bibliography

Coronavirus cases:. Worldometer. (n.d.). https://www.worldometers.info/coronavirus/

Lemon (Citrus limon) Juice Has Antibacterial Potential against DiarrheaCausing Pathogen. Radware bot manager Captcha. (n.d.). https://iopscience.iop.org/article/10.1088/1755-1315/217/1/012023/pdf

Puvača, N., Milenković, J., Galonja Coghill, T., Bursić, V., Petrović, A., Tanasković, S., Pelić, M., Ljubojević Pelić, D., & Miljković, T. (2021). Antimicrobial Activity of Selected Essential Oils against Selected Pathogenic Bacteria: In Vitro Study. Antibiotics (Basel, Switzerland), 10(5), 546. https://doi.org/10.3390/antibiotics10050546

Lontsi Djimeli, C., Tamsa Arfao, A., Noah Ewoti, O. V., Nougang, M. E., Moungang, M. L., Bricheux, G., Nola, M., & Sime-Ngando, T. (2014). Mixture of sodium hypochlorite and hydrogen peroxide on adhered aeromonas hydrophila to solid substrate in water: Impact of concentration and assessment of the synergistic effect. International journal of bacteriology. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4745472/

Truong, W. R., Hidayat, L., Bolaris, M. A., Nguyen, L., & Yamaki, J. (2021). The antibiogram: key considerations for its development and utilization. JAC-antimicrobial resistance, 3(2), dlab060. https://doi.org/10.1093/jacamr/dlab060

Mahdizadeh S;Sawford K;van Andel M;Browning GF; (n.d.). Efficacy of citric acid and sodium hypochlorite as disinfectants against Mycoplasma Bovis. Veterinary microbiology. https://pubmed.ncbi.nlm.nih.gov/32273009/

Drexler, M. (1970, January 1). How infection works. What You Need to Know About Infectious Disease. https://www.ncbi.nlm.nih.gov/books/NBK209710/#:~:text=Sometimes%20bacteria%20multiply%20so%20rapidly,reaction%20that%20is%20itself%20toxic

Centers for Disease Control and Prevention. (2016, September 18). Chemical disinfectants. Centers for Disease Control and Prevention. https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html

Angela Betsaida B. Laguipo, B. (2019, June 14). Does vinegar kill bacteria?. News. https://www.news-medical.net/health/Does-Vinegar-Kill-Bacteria.aspx

Libretexts. (2020, September 24). 22.4: Blood agar plates (BAP). Biology LibreTexts. https://bio.libretexts.org/Courses/College_of_the_Canyons/Bio_221Lab%3A_Introduction_to_Microbiology_(Burke)/22%3A_Physiological_Tests_for_Characterization_and_Identification_of_Bacteria/22.04%3A_Blood_Agar_Plates_(BAP)

Mueller, M. (2023, July 13). Escherichia coli infection. StatPearls [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK564298/

Taylor, T. A. (2023, July 17). Staphylococcus aureus infection. StatPearls [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK441868/

Bell-Young, L. (2023, December 13). The science behind disinfectants: How and why they work. ReAgent Chemical Services. https://www.reagent.co.uk/blog/how-does-disinfectant-work/