TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Comparing the bactericidal effect of white vinegar, chlorine tablets, bleach and Ex'sir solution on bacteria; to clean vegetables in the kitchen environment.

Biology Extended Essay

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Abstract

It is known that, several types of pathogenic bacteria are present in everywhere, including the surfaces of nutrients that people consume every day. Aim of this extended essay is to determine which type of non-toxic cleaning product is more efficient on destroying those bacteria on nutrients, in household usage. In other words, the efficiency of four different cleaning products is compared throughout this study. Therefore, the research question of this study is "Is there a statistically significant difference in bactericidal effects of chosen cleaning products, which are white vinegar, bleach, chlorine table and Ex'sir solution; in terms of their capability of inhibiting bacterial growth on fruits and vegetables?" It is hypothesized that "There will be significant differences in terms of bactericidal effect and inhibiting bacterial growth on fruits and vegetables in between the cleaning products used." Moreover, it is expected that bleach has more efficiency than other cleaning products since it has the most alkaline properties among others.

In order to test the hypothesis stock samples of *E. coli* and *P. aeruginosa* are taken. By two-fold serial dilution technique, the concentrations of four different cleaning products are diluted and bacteria samples are added in those solutions. After overnight incubation, the presence of bacteria in cleaning products, starting from different concentrations, are determined by observing turbidity in the wells. After obtaining the data, Kruskal-Wallis and Bonferroni Dunn tests are used to examine the results of the experiment.

As a result, the results of Kruskal-Wallis test show that there is a significant difference between the products in terms of their bactericidal effects. It is found that chlorine tablet has the highest efficiency among the chosen cleaning products, in means of destroying both types of bacteria present in this study. The efficiency is followed by bleach, Ex'sir sanitizer solution and white vinegar.

Word Count: 300

Abbreviations

MHB: Mueller Hinton Broth (see Appendix 1)

MIC: Minimum inhibitor concentration

ATCC: American Type Culture Collection

PBS: Phosphate-buffered saline

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I. Introduction

"A bacterium is a prokaryotic living cell, consisting of a fluid called cytoplasm enclosed by a cell membrane and cell wall."[1] They were one of the first life forms that live on Earth and they still continue to live their lives in different forms in different mediums. Bacteria can be found in most habitats on Earth such as; growing soil, water, organic matter and also in the live bodies of plants and animals."

However bacterium can be found in nutrients that people consume everyday and some of those bacteria are said to be pathogenic where some are classified as non-pathogenic. Bacteria that cause any kind of illnesses are pathogenic bacteria. Nevertheless, non-pathogenic bacteria do not cause any kind of inflammation in a human's body; moreover they can be used to overcome some allergic reactions or in the process of producing some foods such as yogurt, bread etc.

There are many kinds of bacteria that found on food as; *Streptococcus, Shigella, Campylobacter jejuni, Salmonella, Escherichia coli* and *Pseudomonas aeruginosa.* Those bacteria are determined as pathogenic bacteria that mostly cause some serious illnesses. Some of those illnesses are; *S. typhimurium* infection caused by *Salmonella* bacteria, Guillain-Barré syndrome caused by *Campylobacter jejuni*. Moreover *P. aeruginosa* and *Streptococcus* can lead into pneumonia where *E. coli* bacteria are responsible from urinary tract infection.

The increase in the number of bacteria colonies on the nutrients increases the incidence and prevalence of different illnesses on humans in our century. In recent years, prevalence of foodborne illness which resulted from contaminated food or pathogenic bacteria is increased. This increase has negative impacts on public health. Nowadays there are some ongoing

[1] "Bacterium"
<http://www.bioscience-info.com/bacterium>.

studies about this situation and scientists are trying to manage with those foodborne illnesses by decreasing the bacteria on foods in an efficient way.

There are three important facts about bacteria. First of them is that, they can be killed by using antibiotics, second one is that it is easy to prevent from their impacts and the most important one is that, different types of bacteria can survive in different mediums with different pH ranges. Mediums with pH below 7 is determined as acidic, where above 7 is determined as alkaline.

In this experiment, *E. coli* and *P. aeruginosa* bacteria were used to limit the extent of study. Those two types of bacteria have both pathogenic and non-pathogenic species. However in this research, the pathogenic species will be handled and stock bacteria species will be used.

According to literature survey it is found that the "pH range for optimum growth for *E. coli* bacteria is between 6.7 and 7.5."[2] On the other hand, "the optimal pH range for *P. aeruginosa* bacteria to grow is around 7.3."[3] As a result, both *E. coli* and *P. aeruginosa* bacteria are alkaline-lovers.

Bacteria are present in everywhere including the nutrients that people consume everyday. They can easily spread in places where there is temperature abuse, cross contamination or failures during processing. To avoid from those bacteria and the illnesses they cause, it is important to know the origins of them. Pathogenic bacteria are naturally found in raw food especially in meat, rice and dairy products. Moreover, pests and pets play an important role on spreading those pathogenic bacteria by contaminating foods. Although pests and pets have a role in contamination, people do have an impact as pets on transport of

[2] "Characteristics of Bacteria."
<http://science.jrank.org/pages/714/Bacteria.html>.
[3] "*P. aeroginosa.*"
< http://www.ncbi.nlm.nih.gov/pubmed/18820073>.

bacteria to food. Since people do not care about their hygiene, harmful bacteria find a perfect medium to settle and this situation results in foodborne illnesses in an indirect way. Lastly, the most important and dangerous origin is dust, dirt and mostly soil. Soil contains pathogenic bacteria and it has to be washed out really carefully.

There are several ways to preserve foods and stop the growth of bacteria on nutrients that people consume. In ancient times there were some traditional techniques to prevent foods from bacteria, and today some of those techniques are still in people's lives. "Egyptians dried their foods in sun for preserving them, and after a while, Chinese people used salt, spices and smoking for creating sterile conditions for nutrients."[4] As time passed, people started to use the advantages of the places they live in, and stored and froze their nutrients in high altitudes for preventing them from bacterial growth. As technology improved, nowadays people sterilize the nutrients they consume by using different types of cleaning products.

Bacteria's growth metabolism has a direct relationship with the acidic products, therefore acidity and alkalinity inhibits bacterial growth. Although the highly alkaline products can be more effective than others, acidic products are more frequently used in household usage on nutrients. On the other hand, temperature has an important effect on growth metabolism of a bacteria and an increase in temperature to the optimum value, it can help bacteria to continue to its life at ease.

It is believed that these bacteria can be destroyed by using different bacteriostatic agents which are not harmful for human health. Therefore it was decided to examine and compare the bactericidal efficiencies of different cleaning products. These products can be listed as; water containing one drop of bleach, vinegar, water with chlorine tablet and exsir sanitizer.

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[4] "Early Methods of Food Preservation."
<http://acswebcontent.acs.org/landmarks/landmarks/frozen/fro2.html>.

In Turkey, one of the most traditional cleaning materials for bacteria on food is white apple vinegar. It is commonly used because of its cheap costs, practical usage and its being non-toxic. White vinegar is an acidic liquid which can be used as a household cleaning agent. There are several types of vinegar which can be used for cooking, pickling or some medical processes but for household cleaning usage, the most preferred types are distilled or apple vinegars.

However in recent years, as research opportunities and technology developed, a special sanitizer was started to use to decontaminate pathogenic bacteria or pesticides on fruits and vegetables. This sanitizer is called as Ex'sir solution and since it's 100% herbal and doesn't contain any additives, its household usage and popularity is gradually increasing.

Although highly alkaline products such as chlorine tablet or bleach are not commonly used in household usage, in public restaurants or hotels, they are preferred more than acidic products since their effects are much higher than others. According to researches, one drop of bleach, diluted with sufficient volume of water, is enough to sterilize nutrients and prevent them from pathogenic bacteria. "Bleach has been used since 18th century as a disinfectant which has whitening and color removing effect but it also has strong bactericidal effects and that is the main reason of using bleach in sterilizing".[5]

On the other side, chlorine tablets can be used for both sterilizing water and they are another decontamination method if they are diluted serially in high amounts since they're highly toxic in household usage.

The primary aim of this study is to find out which cleaning product is more efficient in destroying the bacteria in household cleaning of nutrients. Since the prevalence of foodborne illnesses is increasing day by day as technology improves, there have to be some missing

[5] "Bleach." Wikipedia, The Free Encyclopedia. 14 Nov. 2011, 19:19 UTC. <http://en.wikipedia.org/wiki/Bleach>.

points in that situation and the lack of those points can cause serious damages in some people's lives. As it was said before, many scientists are working for preventing pathogenic bacteria from nutrients and it was believed that this experiment will make an important contribution to that area of study.

In this essay bacteriostatic and bactericidal terms were commonly used. "The term bacteriostatic is used to determine an agent that inhibits the bacterium's ability to replicate without killing it. The term bactericidal refers to an agent that directly kills the bacterium or microorganisms, through disrupting its enzyme mechanism or else".[6]

Due to public controversy, many discussions were discussed to determine which one of the bacteriostatic agents has the most efficiency against *E. coli* and *P. aeruginosa* bacteria on nutrients. The main purpose of this study will be based on comparing the efficiencies of different products which are used to clean nutrients as there are several ongoing studies concerning this issue. Therefore, the research question of this study is "Is there a statistically significant difference in bactericidal effects of chosen cleaning products, which are white vinegar, bleach, chlorine table and Ex'sir solution; in terms of their capability of inhibiting bacterial growth on fruits and vegetables?"

[6]"Antimicrobial chemotherapy." 14 Nov. 2011, 21:20 UTC <http://www.cehs.siu.edu/fix/medmicro/chemo.htm>.

II. Hypothesis

Cleaning products which were used in the experiment have acidic or alkaline properties. Among those products; multi-usage bleach and chlorine tablets are alkaline where apple vinegar and Ex'sir are acidic. Furthermore, bleach is the most alkaline one and apple vinegar is the most acidic one among other products.

Bacteria which were used in this experiment can only live within their pH ranges. Therefore too higher or lower pH ranges with respect to the optimum pH can cause them to be destroyed from the medium.

In this study, it is hypothesized that, "There is a significant differences in terms of bactericidal effect and inhibiting bacterial growth on fruits and vegetables in between the cleaning products used." Moreover, it is expected that bleach has more efficiency on destroying the bacteria on vegetables since it has the most alkaline properties and it has the most deviated pH value from the optimum among others.

III. Method Development and Planning

After the selection of four cleaning products was done; an appropriate method was developed to compare the efficiencies of different products according to their bactericidal abilities. It was decided to perform the experiment in laboratory conditions to prevent from any outside contamination.

This experiment was carried out in the laboratories of Baskent University in order to isolate the medium for bacterial growth and to allow the experimenter to identify the relevant bacteria and bacteriostatic agent. Since the collection of bacteria samples from nutrients is difficult, two types of bacteria -*E. coli and P.aeruginosa*- which are commonly found on nutrients were selected and samples were provided by the lab technicians.

In order to compare the efficiencies of four types of cleaning products, provided samples put in different bacteriostatic cleaning agents. After overnight incubation, samples collected and the comparison of the number of colonies of bacteria was made. For each type of bacteria, four trials were made with twelve different concentrations of four different cleaning products. Since the toxic effects of products are different, different concentrations for each product are used. Additionally in each trial, MIC values –which mean the lowest concentration of a solution to inhibit visible bacterial growth- were marked. Rows named A-D symbolized four trials of *E. coli* while E-H was for *P.aeruginosa.* After the incubation, it was expected to see the formation of colonies in the wells. Subsequently the experiment was over, the turbidity of the bacteria and disinfectant mixture showed the efficiency and bactericidal effects of each of the cleaning products.

Since bacteria tend to aggregate instead of spreading in the medium, the forming colonies couldn’t be counted individually. Therefore; the presence of bacteria, in the samples

containing diluted cleaning products, in the wells were analyzed in order to decide which product is more efficient.

Materials Used in the Experiment:

- Ex'sir® Fruit-Vegetable Decontamination Concentration
- Domestos® Multi-Usage Bleach
- Doğanay ® Apple Vinegar
- Klortab® Multi-Usage Chlorine Tablet
- Heraeus® Incubator
- 5 empty test tube
- 4 conical tube containing cleaning products
- 1 conical tube containing Mueller Hinton Broth
- 1000 mL balloon flask containing distilled water
- 4 empty 96-well microwell plates
- *E. coli* American Type Culture Collection (ATCC) 25922 bacteria
- *P. aeruginosa* ATCC 27853 bacteria
- Micro-pipette (100 µL) (Eppendorf, UK)
- Multi-channel pipette (100 µL) (Eppendorf, UK)
- Tips for pipettes
- Gloves
- Lab coat
- Goggles
- Masks

IV. Method

Gloves, mask and lab coat must be worn during the procedure to prevent from the effect of external contamination of the samples.

Procedure:

A. Cultivation and Incubation of Samples

1. Solutions of the cleaning products are diluted according to manufacturer's instructions.
2. Liquid microdilution method was used in 96-well U-based sterile microwell plates.
3. 100 µL MHB was transferred into all plates by using Multi-Channel pipette.
4. Each cleaning agent was transferred into the first wells of each different plate in needed amount for 3 steps.
5. Two fold serial dilutions were done for each cleaning product in each plate. (For details, see Appendix 2.)
6. Last well of each plate was left empty for positive reproduction control.
7. Bacteria were prepared according to McFarland Turbidity Standard No: 0.5. (For details, see Appendix 3.)
8. Prepared bacteria were diluted 100 times by MHB and transferred into each well.
9. All plates are left for 24 hours incubation at 37°C.

B. Identification of Bacteria Growth

1. For identification of the bacteria growth; the turbidity of the wells in microwell plates is very important.
2. The turbidity of the bacteria and disinfectant mixture in the wells determined the bacteria reproduction.
3. The lowest concentration with no reproduction of bacteria was determined as MIC value.

V. Results

Results obtained from the experiment are given in Table 1,2,3 and 4 below.

Table 1: The efficiency of Ex'sir solution on *E. coli* and *P.aeruginosa* bacteria, depending on the bacterial growth in different diluted concentrations of the solution.

Ex'sir Solution		**Concentration of cleaning product**											
		2.25%	1.12%	0.56%	0.28%	0.14%	0.07%	0.03%	0.02%	0.009%	0.004%	0.002%	0.001%
		Well Numbers											
Bacteria Name	**Name of Columns**	1	2	3	4	5	6	7	8	9	10	11	12
E. coli	A				X								
	B				X								
	C				X								
	D				X								
P.aeruginosa	E				X								
	F				X								
	G				X								
	H				X								

X shows the MIC. Above this value, there is no growth of both bacteria.

Table 2: The efficiency of Chlorine tablet on *E. coli* and *P.aeruginosa* bacteria, depending on the bacterial growth in different diluted concentrations of the solution.

Chlorine Tablet		**Concentration of cleaning product**											
		0.25%	0.12%	0.06%	0.03%	0.01%	0.008%	0.004%	0.002%	0.001%	0.0005%	0.0002%	0.0001%
		Well Numbers											
Bacteria Name	**Name of Columns**	1	2	3	4	5	6	7	8	9	10	11	12
E. coli	A							X_1					
	B							X_1					
	C							X_1					
	D							X_1					
P.aeruginosa	E						X_2						
	F						X_2						
	G						X_2						
	H						X_2						

X_1 shows the MIC for *E. coli*. Above this value, there is no growth of bacterium.

X_2 shows the MIC for *P.aeruginosa.* Above this value, there is no growth of bacterium.

Table 3: The efficiency of Bleach on *E. coli* and *P.aeruginosa* bacteria, depending on the bacterial growth in different diluted concentrations of the solution.

Bleach		**Concentration of cleaning product**											
		5%	2.5%	1.25%	0.62%	0.31%	0.16%	0.08%	0.04%	0.02%	0.01%	0.005%	0.002%
		Well Numbers											
Bacteria Name	**Name of Columns**	1	2	3	4	5	6	7	8	9	10	11	12
E. coli	A					X_3							
	B					X_3							
	C					X_3							
	D					X_3							
P.aeruginosa	E				X_4								
	F				X_4								
	G				X_4								
	H				X_4								

X_3 shows the MIC for *E. coli*. Above this value, there is no growth of bacterium.

X_4 shows the MIC for *P.aeruginosa.* Above this value, there is no growth of bacterium.

Table 4: The efficiency of White vinegar on *E. coli* and *P.aeruginosa* bacteria, depending on the bacterial growth in different diluted concentrations of the solution.

White Vinegar		**Concentration of cleaning product**											
		5%	2.5%	1.25%	0.62%	0.31%	0.16%	0.08%	0.04%	0.02%	0.01%	0.005%	0.002%
		Well Numbers											
Bacteria Name	**Name of Columns**	1	2	3	4	5	6	7	8	9	10	11	12
E. coli	A			X									
	B			X									
	C			X									
	D			X									
P.aeruginosa	E			X									
	F			X									
	G			X									
	H			X									

X shows the MIC. Above this value, there is no growth of both bacteria.

V. Data Analysis

Data obtained from the experiment are tabulated in the results section and the analysis of Tables 1,2,3,4 is shown below. Since parametric test assumptions were not available, medians of concentration of cleaning product groups were compared by using Kruskal-Wallis test. [7] Multiple comparisons between pairs of group were carried out according to Bonferroni-Dunn test and p value <0.05 was considered statistically significant.

Kruskal-Wallis Test

H_0: There would not be a statistically significant median difference between groups.

H_1: There would be a statistically significant median difference between groups.

Table 5: Results of Kruskal-Wallis test showing the comparison of medians of concentration of different cleaning products.

Ranks

	GRUP	N	Mean Rank
ECOLI	Bleach	4	10.50
	White Vinegar	4	14.50
	Ex'sir Solution	4	6.50
	Chlorine Tablet	4	2.50
	Total	16	
PAERUGIN	Bleach	4	10.50
	White Vinegar	4	14.50
	Ex'sir Solution	4	6.50
	Chlorine Tablet	4	2.50
	Total	16	

[7] Data analyses were performed using the Statistical Package for the Social Sciences Version 17.0 (SPSS Inc., Chicago IL, USA)

Test Statistics[a,b]

	ECOLI	PAERUGIN
Chi-Square	15.000	15.000
df	3	3
Asymp. Sig.	.002	.002

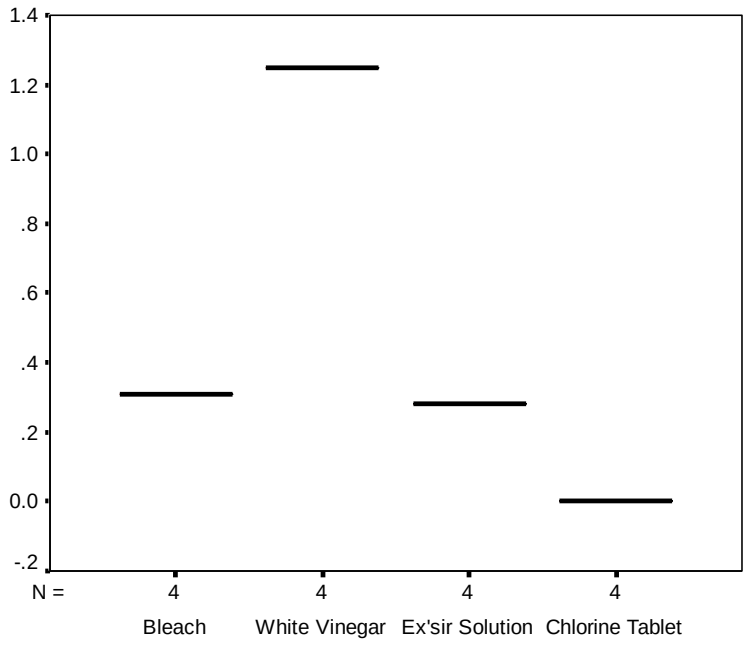
a. Kruskal Wallis Test

b. Grouping Variable: GRUP

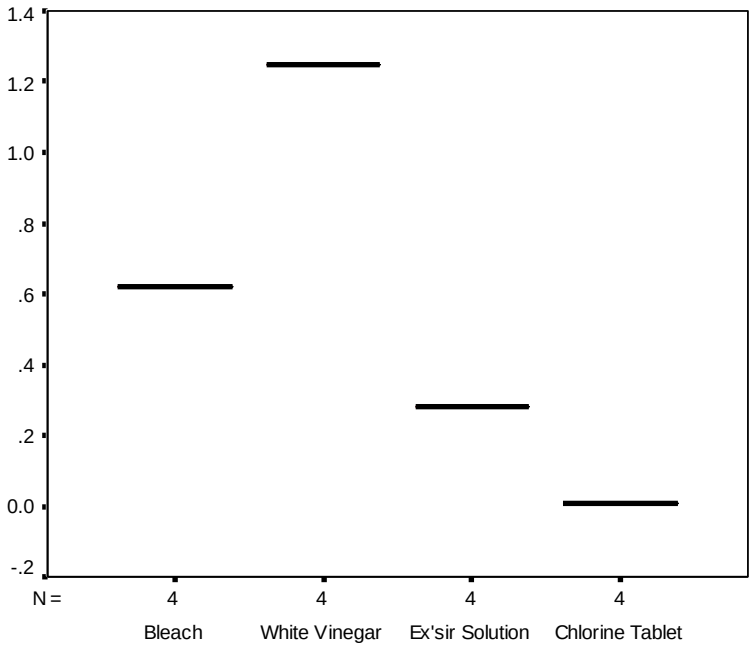
Table 6: Descriptives (mean, N, standard deviation, median, minimum, maximum) of different cleaning products.

Report

GRUP		ECOLI	PAERUGIN
Bleach	Mean	.31000	.62000
	N	4	4
	Std. Deviation	.000000	.000000
	Median	.31000	.62000
	Minimum	.310	.620
	Maximum	.310	.620
White Vinegar	Mean	1.25000	1.25000
	N	4	4
	Std. Deviation	.000000	.000000
	Median	1.25000	1.25000
	Minimum	1.250	1.250
	Maximum	1.250	1.250
Ex'sir Solution	Mean	.28000	.28000
	N	4	4
	Std. Deviation	.000000	.000000
	Median	.28000	.28000
	Minimum	.280	.280
	Maximum	.280	.280
Chlorine Tablet	Mean	.00400	.00800
	N	4	4
	Std. Deviation	.000000	.000000
	Median	.00400	.00800
	Minimum	.004	.008
	Maximum	.004	.008
Total	Mean	.46100	.53950
	N	16	16
	Std. Deviation	.486328	.479200
	Median	.29500	.45000
	Minimum	.004	.008
	Maximum	1.250	1.250



Graph-1: Means and medians of MIC values (which are equal in each product) of different products for *E. coli* bacteria.



Graph-2: Means and medians of MIC values (which are equal in each product) of different products for *P. aeruginosa* bacteria.

VI. Evaluation

In this investigation, the research question was determined as; “Is there a significant difference in terms of bactericidal effect and inhibiting bacterial growth on fruits and vegetables in between the cleaning products used?” Depending on this question, the primary aim of this study was to find out which cleaning product is more efficient in destroying the bacteria in household cleaning of nutrients. It was hypothesized that there is a statistically significant difference in terms of bactericidal effects in between different cleaning products. In all four different products, bleach was expected to have more efficiency on destroying the bacteria on vegetables because of having the most alkaline properties among others.

In Tables 1, 2, 3 and 4, concentration values, which were required for bacteria to start growing, were found for different products. Tables were divided into two sections to show the effect of the same cleaning product on both *P. aeruginosa* and *E. coli* bacteria colonies and twelve columns were used to show the decreasing concentrations of diluted cleaning products from left to right.

It can be seen in Table 1 that, Ex’sir solution has the same effect on both types of bacteria and two colonies can survive and grow in 28% diluted concentration of Ex’sir solution and that concentration was determined as the maximum diluted concentration for bacterial growth of *P. aeruginosa* and *E. coli*.

Table 2 shows the results obtained for chlorine tablet. It can be said that chlorine tablet was more efficient than Ex’sir solution in destroying the bacteria since the maximum diluted concentration for bacterial growth of *P. aeruginosa* is 0.008% while it is 0.004% for *E. coli.* Moreover, those results also exposed that chlorine tablets are more efficient on *E. coli* bacteria than *P. aeruginosa.*

The results for bleach were given in Table 3 showing that bleach is more efficient on *E. coli* bacterium rather than *P. aeruginosa.* Besides, the maximum diluted concentration for bacterial growth of *P. aeruginosa* was found as 0.62%, while it was found as 0.31% for *E. coli.* Hence it can be said that bleach is more efficient that Ex'sir solution.

Furthermore in Table 4, it can be observed that the efficiency of white vinegar on inhibiting bacterial growth of both *E. coli* and *P. aeruginosa* is the same, which is 1.25%. Hence it can be said that white vinegar is the less efficient cleaning product on destroying the bacteria on nutrients.

To interpret the obtained results and understand whether there is a statistically significant difference between groups or not, two statistical analyses were done. The first one is Kruskal-Wallis test, which the medians of each group were compared. In this experiment, since the concentrations for every group of cleaning product was different, parametric test assumptions were not available. Hence, the medians and means of every group of products were found as equal and according to the result in Table 5; p-value was found to be 0.002 for each bacteria, which is a smaller value than $\alpha = 0.05$. Therefore the H_0 hypothesis was rejected, and H_1 hypothesis, implying that there would be a statistically significant difference between groups was supported. Moreover, Bonferroni-Dunn test was also performed to make multiple comparisons between pairs of groups. Due to the analyses performed in the experiment, main hypothesis was partially accepted. However; a different product, instead of chlorine tablet, was expected to have more efficiency on destroying bacteria on nutrients. According to the results of the experiment, efficiency of products can be given from highest to the lowest as; chlorine tablet, bleach, Ex'sir and vinegar.

Although those results partially supported the main hypothesis of the experiment, following improvements or changes can be done to decrease the possibility of errors and make the results more precise.

- Depending on literature survey, it was accepted that; during the experiment, the effect of products was tested on *E. coli* and *P. aeruginosa* bacterium. However in real life, as it mentioned before, there can be other bacteria colonies formed on nutrients. To avoid from errors which this can cause, samples could be collected directly on some vegetables instead of using stock bacterium.

- Although quantitative data was collected by looking at the medians of concentrations of different groups, bacterial growth was determined by observing turbidity, which accounts as qualitative observations. Since the turbidity determined whether there was bacterial growth or not, the significance of the results can change. To prevent deviations, agar-plate sampling could be used and number of colonies formed could be counted.

VII. Conclusion

In this experiment; the bactericidal effect of white vinegar, chlorine tablets, bleach and Ex'sir solution on bacteria was compared in means of their efficiency to clean vegetables in the kitchen environment. As stated before, the primary aim of this study was to find out which of the listed cleaning product is more efficient in destroying the bacteria in household cleaning of nutrients. Therefore it was hypothesized that "There is a significant differences in terms of bactericidal effect and inhibiting bacterial growth on fruits and vegetables in between the cleaning products used."

According to the results of the experiment, efficiency of products can be given from highest to the lowest as; chlorine tablet, bleach, Ex'sir and vinegar. Before starting the experiment, based on public belief, it was expected that bleach would be the most efficient product but that expectation was rejected due to the results of the experiment. It is known that chlorine-based halazon tablets were commonly used in our century and during the World War II for portable water purification.[8] Furthermore, it can be said that, chlorine tablet usage is more recommendable than bleach usage in cleaning nutrients.

However, when recommending a cleaning agent for nutrients, the cost of the product and its toxic effects also has to be considered. Since chlorine tablet is the one which has the most toxic effects on people when it is used inappropriately or diluted differently from the constructer's instructions; the usage of it in household usage can cause undesirable effects. Moreover chlorine tablets are mostly used as disinfectant for swimming pools, their cost are set for high numbers and it is also difficult to find it in small amounts as a household cleaning product for nutrients. Although it is known that chlorine tablets are not preferably used by people in the whole world, chefs of some restaurants or hotels generally use it to save time.

[8]"Portable water purification." Wikipedia, The Free Encyclopedia. 14 Nov. 2011, 20:31 UTC. <http://en.wikipedia.org/wiki/Chlorine_Dioxide_Tablets#Chemical_disinfection>.

When all the toxic effects, costs and people's opinions are considered, it is understood that the usage of chlorine tablet and bleach is not a suitable way of cleaning nutrients. Therefore, Ex'sir solution and vinegar are said to be more convenient in household usage as a cleaner. Although the results show that Ex'sir solution has a higher efficiency of destroying bacteria than vinegar, the difference is not much. However, when the advertisements about the Ex'sir solution is considered, it can be said that those advertisements deceive people by frightening them about the bacteria they live with and by defaming vinegar usage by saying that it is not as efficient as Ex'sir solution, in destroying the bacteria in kitchen environment.

Although there is no literature source about the prevalence of the usage of vinegar as a cleaner on nutrients, depending on public beliefs and traditions; it can be said that vinegar did well on destroying the bacteria on nutrients from past until today. Moreover, by looking at the results; the effect of vinegar can't be ignored. Since vinegar doesn't contain any extra additives, it is recommended for household usage on nutrients rather than Ex'sir solution which contains different fruit extracts. Hence, vinegar was determined as the most convenient cleaning product to use in household usage to inhibit bacterial growth on nutrients.

To sum up, the reason which lies behind why this subject is chosen for this study was to evaluate which type of bacterium are generally found on nutrients that people consume every day, to figure out the effects of those bacterium on human health and to determine which type of cleaning product is more efficient on destroying bacteria or inhibiting their bacterial growth on nutrients. As the research question is answered, aim is achieved and hypothesis is partially accepted; it is consulted that this survey is succeeded. Although public beliefs about using which cleaning product in household usage will not change abruptly; it is believed that the results obtained from this research will be effective in the determination process. Nonetheless, as technology improves and new techniques develop, debates over which cleaning product should be used on nutrients will continue.

VIII. Appendices

A. Appendix 1

The following is the explanation of Mueller Hinton Broth, its usage and its preparation. (taken from "Oxoid" Website):

MUELLER-HINTON BROTH

"An antimicrobial susceptibility testing medium which may be used in internationally recognized standard procedures."

Typical Formula*	***gm/liter***
Beef, dehydrated infusion from	300.0
Casein hydrolysate	17.5
Starch	1.5

pH 7.3 ± 0.1 at 25°C

*** Adjusted as required to meet performance standards**

Positive controls:	**Expected results**
Escherichia coli ATCC® 25922 *	Turbid growth
Pseudomonas aeruginosa ATCC® 27853 *	Turbid growth
Enterococcus faecalis ATCC® 29212 *	Turbid growth
Negative control:	
Uninoculated medium	No change

*** This organism is available as a Culti-Loop®**

B. Appendix 2

"The following is a brief explanation of two-fold serial dilution technique which was used in the preparation of different diluted concentrations of different cleaning products. (taken from "Food and Agriculture Organization of the United Nations" Website):

"A two-fold dilution reduces the concentration of a solution by a factor of two that is reduces the original concentration by one half. A series of two-fold dilutions is described as two-fold serial dilutions. In this manual, two-fold serial dilutions are carried out in small volumes in microwell plates. They are used in both the haemagglutination and haemagglutination inhibition tests to establish titres of the test samples."

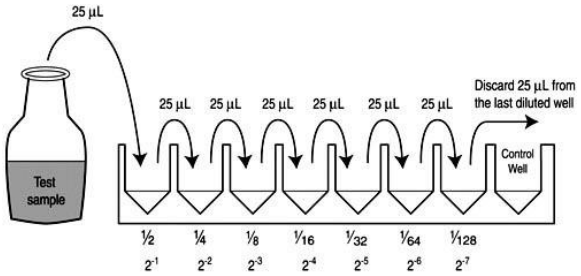
How to make two-fold dilution?

Step 1: Use the micropipette to dispense 25 mL of PBS diluent to the first well.

Step 2: Use the micropipette to transfer 25 mL of the test solution to the first well.

Step 3: Use the micropipette to mix by drawing up the liquid and expelling it again. Carry out this action twice.

Step 4: The well now contains 25 mL of the original test solution diluted by one half in a total volume of 50 mL.



C. Appendix 3

The following is taken from "Pro-lab Diagnostics" Website and McFarland, J., J.Amer.Med.Assoc. **14:**1176, 1907:

Intended Use

The McFarland Equivalence Standards are intended to be part of a quality control program for adjusting densities of bacterial suspensions that are used for identification and susceptibility testing. Each standard is made from different concentrations of latex beads mixed in a buffer liquid. The original McFarland Standards were made from the combination of Barium chloride and Sulfuric acid that result in a flocculate. Problems were encountered with this technique which included instability, storage, and reproducibility of the resulting suspension. These problems have been overcome by using latex particles in a buffer solution to make Colorimeter and McFarland Standards

Principle of the Procedure

The McFarland Equivalence Standards are used for adjusting densities of bacterial suspensions.

Reagents:

McFarland Turbidity Standard No. 0.5

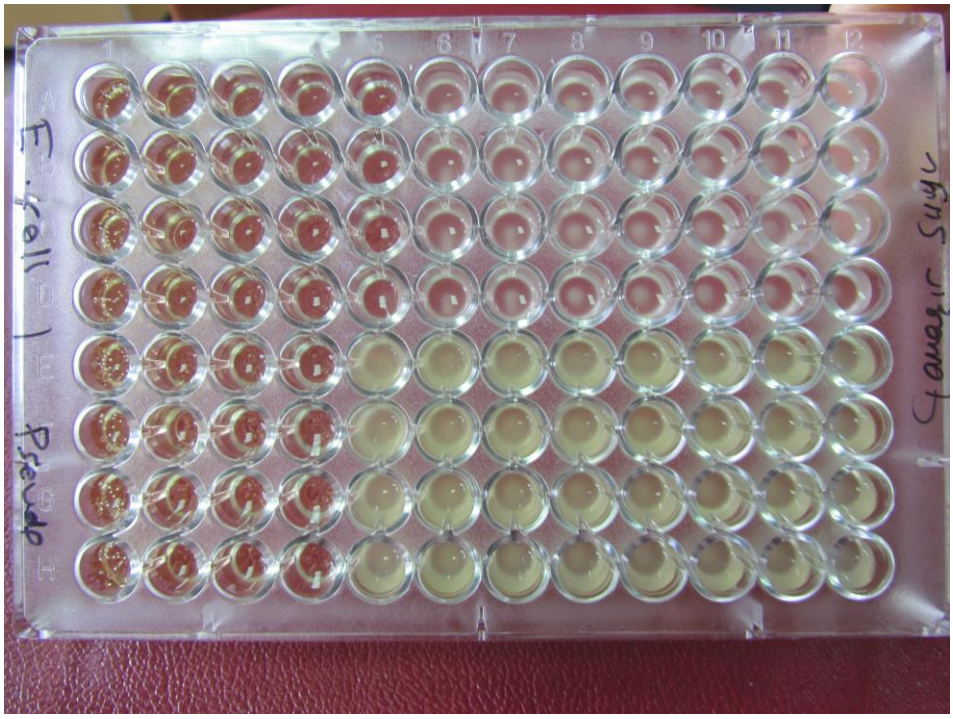
Approximate Formula Per 100 mL Purified Water

Sulfuric Acid, 0.18 M	99.5 mL
Barium Chloride, 0.048 M	0.5 mL

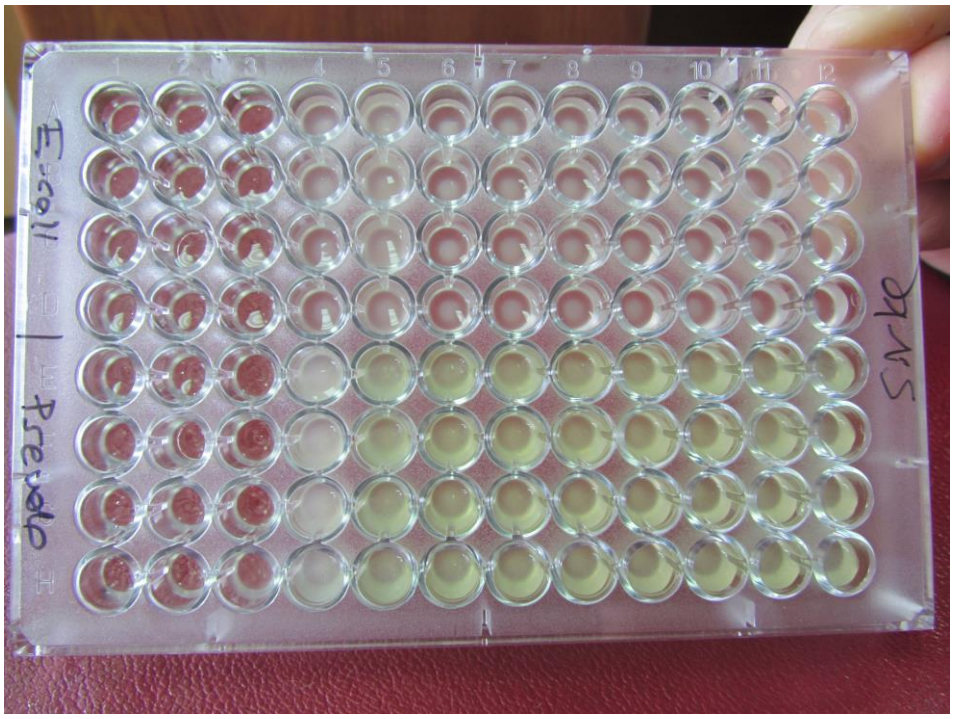
D. Appendix 4

The following are the photos of test tubes containing different concentrations of cleaning products and the photos of the results in 96-well plates for four types of cleaning product.

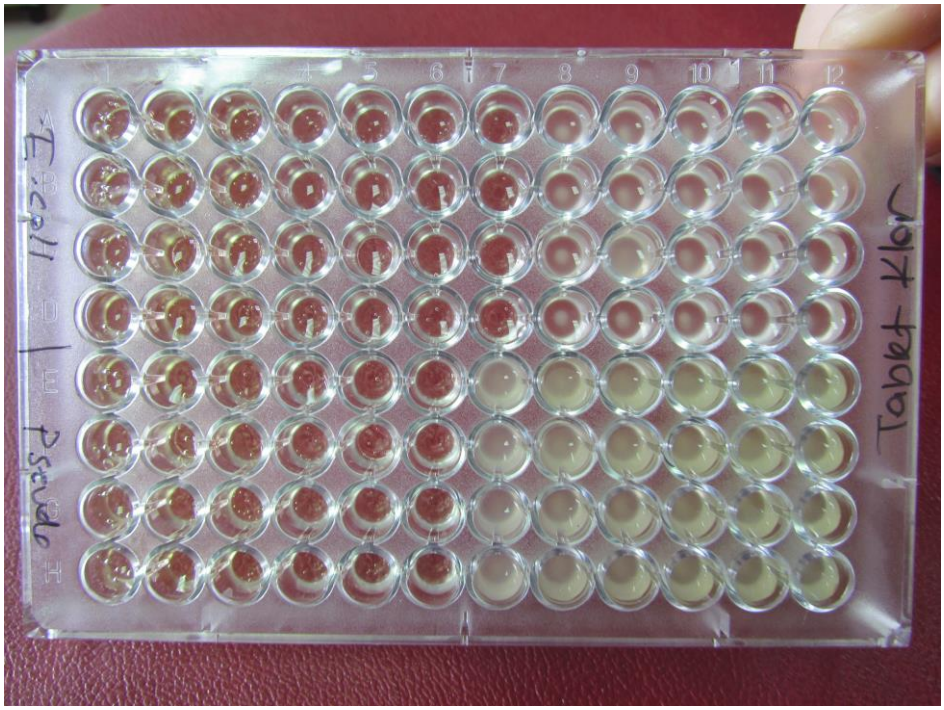
Picture 1: Different concentrations of different cleaning products. From left to right: Chlorine tablet, Ex'sir solution, white vinegar, control solution and bleach



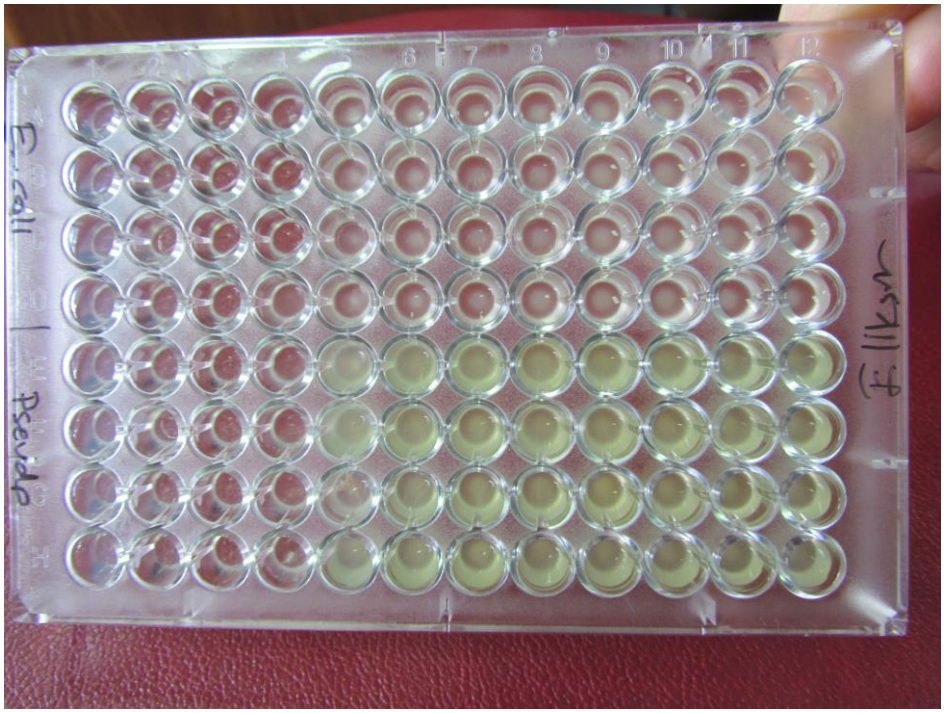
Picture 2: Seem of bacterial growth of *E. coli* and *P. aeruginosa* under the effect of bleach, in 96-well plates.



Picture 3: Seem of bacterial growth of *E. coli* and *P. aeruginosa* under the effect of vinegar, in 96-well plates.



Picture 4: Seem of bacterial growth of *E. coli* and *P. aeruginosa* under the effect of chlorine tablet, in 96-well plates.



Picture 5: Seem of bacterial growth of *E. coli* and *P. aeruginosa* under the effect of Ex'sir solution, in 96-well plates.

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