TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Comparison of the bactericidal effects of medical-level antiseptics used in medical institutions, commercial antibacterial soaps and regular commercial soaps on *Staphylococcus aureus*.

Biology Extended Essay

Supervisor: Sevim SARAL

Name of student: Tevfik Umut DİNÇER

Candidate number: D1129026

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Abstract

The aim of this extended essay is to investigate the bactericidal properties of regular soap, antibacterial soap, and medical-level soap (that is used before surgeries) on the bacterium *Staphylococcus aureus* in lab conditions.

My research question was: “Is there a significant mean difference among antiseptics used in medical institutions, commercial antibacterial soaps and regular commercial soaps in terms of their bactericidal effects on Staphylococcus aureus in laboratory conditions?”

It was hypothesized that; there would be a significant mean difference in terms of efficiency between the groups. The medical-level antiseptic will be the most effective bactericidal agent against *Staphylococcus aureus*, followed by the commercial antibacterial soap, and finally the regular commercial soap.

In order to test the hypothesis and to answer the research question, the Kirby-Bauer antibacterial testing method was used. *Staphylococcus aureus* population was cultured on Mueller-Hinton II agar plate in laboratory conditions. Filter paper discs soaked in solutions of antiseptics used in medical institutions (“medical-level soaps”), commercial antibacterial soaps and regular commercial soaps were inserted onto the agar. The diameters of the exclusion zones were compared. Data analysis is done to determine if there is a significant difference in terms of bactericidal property in between the groups.

Resultantly, the filter paper disc soaked in medical-level soap formed the zone of exclusion with the largest diameter. It is followed by filter paper disc soaked in the commercial antibacterial soap. The filter paper disc soaked in the regular soap had the smallest diameter. ANOVA results revealed that there was a significant mean difference between medical-level soap, commercial antibacterial soap and regular soap in terms of their bactericidal effect on *Staphylococcus aureus*, with the medical-level soap being most effective, followed by commercial antibacterial soap, and regular soap the least effective in comparison.

Word Count: 290

Table of Contents

I. Introduction/Background

Soap has been used by humanity since dates back around 2800 BC in ancient Babylon for non-cleansing purposes such as preparation of wool for weaving[1]. It was first used to maintain hygiene in the 7th century by the Islamic world. Soap gained widespread use only in the late 19th century in the western civilization as its relationship with health and cleanliness became popular. The first antibacterial soap was produced in the United States in the 1950s (Turner), kept gaining a larger market share till today.

Soap functions through the molecule's hydrophilic and hydrophobic ends. When dissolved in water, the hydrophobic end (nonpolar end) sticks to dirt and bacteria, while the hydrophilic end is in water. Thus, soap molecules envelope the dirt particle or bacterium and put them into suspension in the water. When water is washed away, so are the dirt and bacteria.

Antibacterial soaps have an extra active ingredient (usually triclosan, triclocarban or chlorhexidine) that kill bacteria by disrupting its enzyme mechanisms or membrane structure (Kuyyakanond and Quesnel).

Antibacterial soaps have been gaining market share in the soap industry since the 1960ties. One of the main reasons of this gain of market share is the extensive advertising campaign that suggested that even though antibacterial soaps were more expensive, they are what must be bought by concerned mothers to protect their children. There are various studies on the question: "is antibacterial soap more effective than regular soap?" (Aiello, Larson ve Levy)

Triclosan-containing soaps were subject to much debate over the past ten years. It was claimed that antibacterial soap is not only not better than regular soap in ter ms of health benefits; it contained a risk of creating a generation of bacteria that are resistant to bactericidal agents, so are harder to kill. Most studies on this topic in community setting came to the conclusion that triclosan containing commercial soaps available to the general public were no better than regular soaps (Aiello, Larson ve Levy) [4] in terms of reducing infections, and several studies on bacteria developing resistance to triclosan concluded positive in laboratory setting.

In hospitals, chlorhexidine based hand cleaning agents and alcohol-based hand cleaning agents are mainly used before surgeons attend surgeries. A study on dental plaque (which is entirely formed by certain bacteria[2]) buildup suggests that in comparison,

[1] "Soap." *Wikipedia, The Free Encyclopedia*. 24 Dec 2008, 20:59 UTC. 25 Dec 2008 <http://en.wikipedia.org/w/index.php?title=Soap&oldid=259959497>.
[2] "Dental plaque." *Wikipedia, The Free Encyclopedia*. 18 Dec 2008, 03:46 UTC. 18 Dec 2008 <http://en.wikipedia.org/w/index.php?title=Dental_plaque&oldid=258718766>.

chlorhexidine based agents are more effective in killing bacteria than triclosan based agents (Renton-Harper, Addy ve Moran). As these agents are more expensive, they are not commonly used in consumer level products.

The topic of this research is comparing of the bactericidal effects of medical antiseptics used in medical institutions, commercial antibacterial soaps and regular commercial soaps on *Staphylococcus aureus*.

Due to public controversy, many studies were done to reveal if antibacterial soap was better than regular soap, but I was not able to find any research done on comparison of professional cleaners used in medical establishments with regular soap and antibacterial soap. This is why I choose this topic for my extended essay: in this research, it is my aim to make a distinction between the antibacterial properties of commercial antibacterial soaps, regular soaps and professional cleansers used in medical establishments by measuring the diameters of exclusion zones in *Staphylococcus aureus* culture on agar plates.

Only one type of bacterium was used as the subject to limit the extent of this study, whereas it is possible that other bacteria may have different reactions to the experiment. The bacterium *Staphylococcus aureus,* which is commonly used in clinical experiments[3], is chosen for this experiment. The reason *Staphylococcus aureus* was chosen as the subject is that it is a common commensal species of bacterium on our skin, and have pathogen behavior when defensive barriers of the person is weak or breached (e.g. a wound) and cause an infection. Every year, 500.000 patients in US hospitals are infected by Staphylococci. (National Institute of Allergy and Infectious Diseases (NIAID))

The terms bactericidal and bacteriostatic are used throughout the essay. The term bactericidal refers to any agent that directly induces the death of the bacterium, through disrupting its enzyme mechanisms or else. The term bacteriostatic is used to refer to an agent that does not cause the death of the bacterium, but instead, an agent that blocks the bacterium's ability to replicate. (Britannica)

[3]"Staphylococcus aureus." *Wikipedia, The Free Encyclopedia*. 20 Dec 2008, 18:25 UTC. 12 Dec 2008 <http://en.wikipedia.org/w/index.php?title=Staphylococcus_aureus&oldid=259200397>.

II. Hypothesis

Regular soap has been used as the basic tool to maintain personal hygiene for more than a thousand years. Although it is not directly bactericidal, the regular commercial soap may provide a limited bacteriostatic effect: as it puts the bacteria nearby to emulsion in the water by wrapping the bacterium with its hydrophobic ends. Thus, it will cut off its contact with nutrients which are necessary for bacterial reproduction.

The antibacterial soap includes Triclosan, a bactericidal agent, in its ingredients. The antibacterial soap also has all the bacteriostatic properties of regular soap as well.

The medical-level antiseptic contains chlorhexidine, which is a highly efficient bactericidal agent. This agent is frequently used before surgeries, where there is a high risk of infection, by the surgeons to sterilize their hands. One study on comparison of triclosan and chlorhexidine containing products on dental plaque (which is entirely composed of bacteria[4]) revealed that chlorhexidine was more effective against dental plaque than triclosan. (Renton-Harper, Addy ve Moran)

In light of this information, it was hypothesized that there will be a significant mean difference in terms of efficiency in between medical-level soap, commercial antibacterial soap and regular commercial soap. The medical-level soap will be the most effective against *Staphylococcus aureus*, followed by the commercial antibacterial soap, and finally the regular commercial soap.

It is worth noting that the extent of this study is limited to any bactericidal or bacteriostatic effects of regular, antibacterial and medical-level soaps on *Staphylococcus aureus* only. No generalization on the general effect of these soaps on bacterium is done in this study. Also, this study does not reflect any statements that can be made on the effect of using different kinds of soaps on disease frequency of the community, i.e. the effect of using antibacterial soaps to reduce the spread of disease.

[4] "Dental plaque." *Wikipedia, The Free Encyclopedia*. 18 Dec 2008, 03:46 UTC. 18 Dec 2008 <http://en.wikipedia.org/w/index.php?title=Dental_plaque&oldid=258718766>.

III. Method

Development and Planning

The Kirby-Bauer antibiotic testing method will be used in this experiment to compare the bactericidal effect and bacteriostatic effect of regular, antibacterial and medical-level soaps. This method makes use of the diffusion of particles in agar. The area nearest to the source of the diffusing chemical will have the highest concentration. The greater is the distance from the source of diffusion, the smaller the concentration of the chemical.

The main principle of the method is that, an antibiotic that is still effective against bacteria in lower concentrations than the other antibiotics is a stronger antibiotic. Likewise, the soap that has stronger bactericidal (or bacteriostatic) property is a more effective soap, in theory.

The zone of exclusion (see Appendix 1) is the circle of clearness around the source of the bactericidal agent. In this area, no bacterial reproduction or protein synthesis is present because of the effectiveness of the agent. This is the reason of clearness in that area. The exclusion extends as far as the agent is still effective. When the concentration of the agent is reduced down to the limit in which bacteria can live and reproduce in, the clearness ends.

As a result, the greater the radius of the zone of exclusion is, the more effective the agent. Thus, the dependent variable of this experiment is the diameter of the zones of inhibition surrounding the filter paper soaked in different possible antibacterial agents. These are regular soap, antibacterial soap, and medical level soap. The type of soap is the independent variable of this experiment.

The primary reason why I have chosen this method is that this method will provide me with quantitative data for statistical analysis of my results of this experiment, the type of soap used. Another reason is that this method is frequently used by researchers because of its high accuracy, and it was easy to acquire knowledge about this method.

The Kirby-Bauer antibiotic testing method, which is utilized in this experiment, is clinically used for bactericidal testing only, no studies were found using this method to test bacteriostatic properties of an agent. But I expect that this method will work for bacteriostatic properties of agents as well, because when bacteria are put into a suspension in water, surrounded by soap molecules, they would lose contact with the nutrients they need to reproduce. In that case, a zone of exclusion would be present on the petri plate around the filter papers soaked in agents with bacteriostatic properties (such as regular soap).

While selecting the brands of antibacterial and regular soap to be used in this experiment, the criteria was that the brand to be publicly renowned and entirely on-purpose (e.g. clearly labeled as antibacterial). The medical-level soap and solutions chosen are chosen among the industry standards. Protex™ by Colgate-Palmolive Company, which is chosen as the antibacterial soap sample, is widely used in Turkey and dominates the antibacterial soap sector to such extent that the brand name has become identical to the concept of antibacterial soap. Duru is the market leader brand in Turkey whose manufacturer has been producing and marketing regular soap since 1927[5]. Manusprey™ is a chlorhexidine based bactericidal solution that is frequently used by surgeons of Bayındır Hospital in Turkey.

Staphylococcus aureus to be used in the experiment should not have developed any resistance to any of the bactericidal agents above.

The soaps used were purposefully selected liquid soaps to eliminate any concentration variability. Even if the concentrations are different, it would not affect the results of the experiment, as it is the same concentration that is used by consumers. It is not the active ingredients that are compared in this experiment, the soaps themselves.

Staphylococcus aureus was grown on Mueller Hinton II (BBL™) agar (see appendix 2). It is the commonly used agar to be used in the Kirby-Bauer method. The usage of this agar is recommended while working with *Staphylococcii* bacteria as well[6]. The nutritional plate includes beef extract, acid hydrolysate of casein, starch and agar for maximal bacterial growth.

For all groups and trials, the petri dishes were all impregnated with the same number of bacteria of 0.5 McFarland[7] (see Appendix 2), which is the standard procedure for the Kirby-Bauer method.

The conditions inside the incubator must be the same for all groups and trials to avoid any contribution of factors other than the independent variable.

As there is no actual hand washing involved, the extent of this study is only limited to bactericidal and bacteriostatic properties of the agents on bacteria colony in agar. Other studies (Leyden, McGinley ve Kaminer) (Larson E) (Bendig) have attempted to extend the scope of the experiment by better mimicking real life situations: such as the number of bacteria left on the hands after consecutive washes with regular soap and antibacterial soap. Other comprehensive studies (Larson, Lin ve Gomez-Pichardo) (Luby, Agboatwalla ve Painter) investigate the number of infections detected in the community. Families with different socioeconomic classes and different genetic backgrounds are chosen as the

[5]http://www.evyap.com.tr/tr/kurumsal/kurumsal.asp
[6]http://www.bd.com/ds/productCenter/221177.asp
[7] The amount of initial bacteria planted to the agar is measured in McFarlands (See appendix 2).

experiment subjects. The experimental group was given antibacterial soap, and the control group was given regular soap without labels. After washing with antibacterial soap and regular soap, the numbers of infections in the groups were compared. The results were found to be not statistically significant.

I expect that, at the end of the experiment, the exclusion zone near the medical-level soap will have the largest diameter, followed by the sample from the commercial antibacterial product, and finally the regular commercial soap.

Materials used in the experiment:

- 300 ml Protex™ antibacterial soap by Colgate-Palmolive®
- 300 ml Manusprey® biocide solution by Anios Laboratories
- 300 ml Duru® Sıvı El Sabunu (regular liquid hand soap)
- 4 × 90 mm Petri dishes Mueller-Hinton II (BBL™) nutritional agar
- 25 × Standard sized filter papers
- Pure strain non-antibiotic resistant *Staphylococcus aureus*
- Distilled water
- Heraeus series 6000 Incubator
- Millimetric ruler (uncertainty: ±0.5mm)

IV. Method

Mask and gloves must be worn during the procedure at all times to minimize the effect of external contamination to the agar plates.

Procedure:

A. Mueller-Hinton II nutritional agar is prepared:

See Appendix 3.

B. Antibacterial agent impregnated filter papers are prepared:

1. The antibacterial liquid soap (not diluted) is poured into a Petri dish until the Petri dish is full of the soap.
2. Step one is repeated for the regular liquid soap and the Medical-level antibacterial solution.
3. Standardized filter papers are impregnated in the soaps for 15 minutes to allow absorption of soaps by filter papers.

C. Experiment is conducted:

1. Filter papers are inserted into the Mueller-Hinton II agar (five in each agar plate), by the use of gloves, mask and pincers.
2. The top of the Petri dish is shut.
3. The Petri dishes are put into the incubator set to 37°C for 12 hours.
4. The diameters of the exclusion zones are measured and recorded.

V. Results

The table below represents the antibacterial effects of regular soap, antibacterial soap and Medical-level soap by means of the diameters of inhibition zones obtained.

Table 1: The diameters of inhibition zones obtained after 24 hours of incubation of standard *Staphylococcus aureus* samples.

Trials / Type of Soap	Zone Diameters (±1mm)				
	X1	**X2**	**X3**	**X4**	**X5**
Regular Soap	19	21	18	21	17
Antibacterial Soap	23	26	26	28	29
Medical-Level Soap	29	32	31	29	34

VI. Data Analysis

The following formulas were used to obtain the corresponding values[8]:

Mean:

$$\bar{x} = \frac{1}{n} \cdot \sum_{i=1}^{n} x_i$$

where
n is the number of trials (in this experiment 5 for all groups)
x_i is the amount of oxygen trapped in the graduated cylinder for trial number i

Standard Deviation:

$$\sigma = \sqrt{\frac{1}{N}\left(\left(\sum_{i=1}^{N} x_i^2\right) - N\overline{x}^2\right)}$$

$$= \sqrt{\frac{1}{N}\left(\sum_{i=1}^{N} x_i^2\right) - \overline{x}^2}.$$

where
n is the number of trials (in this experiment 5 for all groups)
x_i is the amount of oxygen trapped in the graduated cylinder for trial number i
$\bar{x}$ is the mean value for the corresponding group

Standard Error:

$$SD_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

where
n is the number of trials (in this experiment 5 for all groups)
$\bar{x}$ is the mean value for the corresponding group
σ is the standard deviation of the corresponding group

[8] Formula images were taken from the English version of Wikipedia.

Table 2: The mean values, standard deviations and standard errors of the diameters of exclusion zones around filter papers soaked in regular soap, antibacterial soap and medical-level soap.

Groups	*Count*	*Sum*	*Average*	*Variance*	*Standard Deviation*
Regular Soap impregnated filter papers	5	96	19.2	3.2	1.79
Antibacterial Soap impregnated filter papers	5	132	26.4	5.3	2.30
Medical-Level Soap impregnated filter papers	5	155	31	4.5	2.12

Table 3: Single factor Analysis of Variance (ANOVA) statistical calculation for all groups.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	353.7333	2	176.8667	40.81538	4.43E-06	3.885294
Within Groups	52	12	4.333333			

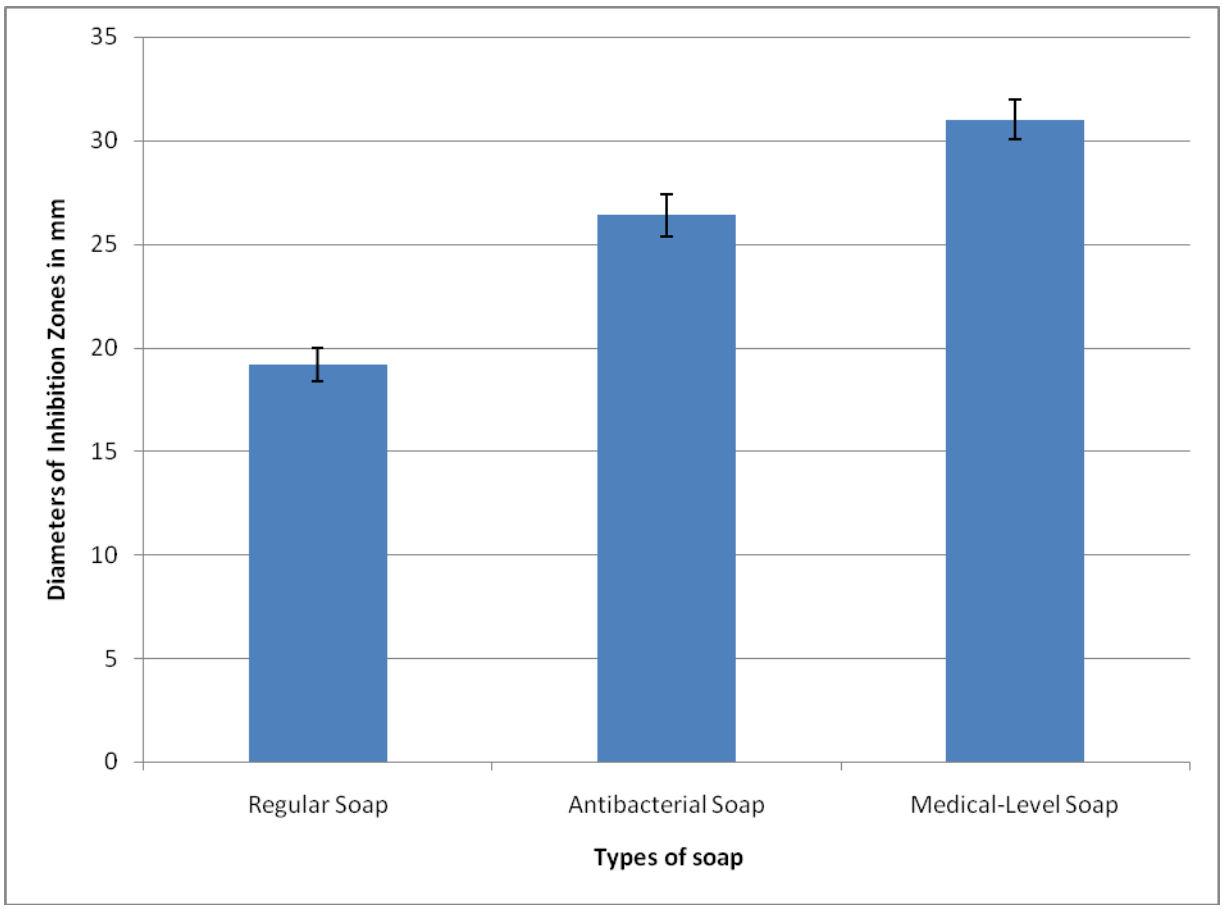
Table 4: T-test: Pair-wise calculation of p-values in order to determine significance of the difference.

Matching of groups	P-value	Existence of significant difference (P<0.05)
Regular soap group versus anti-bacterial soap group	0.000559	Yes
Regular soap group versus medical-level soap group	1.23E-05	Yes
Anti-bacterial soap group versus medical-level soap group	0.011093	Yes

Table 5: Relevant Descriptive Statistics for each experimental group. The data below are obtained using Microsoft Office Excel 2007.

Groups / Parameter	Regular Soap impregnated filter papers	Antibacterial Soap impregnated filter papers	Medical-Level Soap impregnated filter papers
Mean	19.2	26.4	31.0
Standard Error	0.80000000	1.029563014	0.948683298
Standard Deviation	1.788854382	2.302172887	2.121320344
Count	5	5	5
Confidence Level (95.0%)	2.221156084	2.858525191	2.633967099

Graph 1: The comparison of the inhibition zones obtained from regular soap, antibacterial soap, Medical-level soap. The error bars indicate standard error for each group.



VII. Evaluation

The aim of this study was to find out whether there was a significant mean difference in terms of bactericidal property between regular soap, antibacterial soap, and medical-level soap on the bacterium *Staphylococcus aureus* in lab conditions. It was hypothesized that there would be a significant mean difference in terms of efficiency in between the groups, and that medical-level antiseptic would be the most effective against *Staphylococcus aureus*, followed by the commercial antibacterial soap, and finally the regular commercial soap.

The regular soap showed the least antibacterial effect, followed by the antibacterial soap, and Medical level soap has the strongest effect. The diameters of exclusion zones ranged between 17 to 21 mm with the mean value of 19.2 mm for regular soap, 23 to 29 mm with the mean value of 26.4 mm for antibacterial soap and 29 to 34 mm with the mean value of 31.0 mm for the Medical-level soap. These ranges are typical of Kirby-Bauer antibiotic testing method.

My null hypothesis was that there was no significant mean difference between regular soap; antibacterial soap and medical-level soap in terms of their diameter of zone of inhibition in Petri dish planted with *Staphylococcus aureus*. As the p values of pair-wise comparisons of the groups calculated[9] were found to be smaller than 0.05, my null hypothesis was rejected: There is a significant mean difference between each and every group in terms of bactericidal activity (see Table 4).

My hypothesis, which was, “the medical-level antiseptic will be the most effective against *Staphylococcus aureus*, followed by the commercial antibacterial soap, and finally the regular commercial soap” has been supported by the results of the experiment and data analysis (see table 5).

One aspect of the results that was not expected is the relatively high bactericidal effect of the regular soap. I did not expect that the exclusion zones of regular soap group would be comparable to the exclusion zones of the other groups. It seems that in my estimations, the baceriostatic effect of regular soap was highly underestimated.

All groups have apparent differences in the rate of their antibacterial activities. The antibacterial soap is clearly more efficient in halting the reproduction of bacteria in a larger area. The Medical-level soap, on the other hand, is more efficient than both.

The standard deviation values of antibacterial soap and Medical-level soap are slightly higher than the standard deviation value of regular soap. (2.30 and 2.12 and 1.79) This occurrence is likely to be a random variation.

[9] With ANOVA (Analysis of Variance)

During the experiment, there were no unexpected occurrences that may have affected the results of the experiment. However, while writing this essay, I realized some possible systematic errors in the method that may have had affected the results. These are listed below with suggestions for future repetitions:

1. The viscosity of the soaps. Although no obvious differences in their viscosity were observed during all parts of the experiment, no formal viscosity measurement was done with specialized viscosity measuring equipment. Soap with a higher viscosity value may have diffused into a wider region, resulting in a larger zone of exclusion even though the agent itself is not that efficient against bacteria. The experiment can be repeated with solutions of equalized viscosities through dilution.
2. The concentration of the soaps. The soaps may have been optimized for domestic use with sufficient water. In their over-concentrated forms, they may not be as effective as they normally are. Or the converse, the over-saturation of the soap may have caused a stronger bactericidal effect, while the regular usage may not be as effective. The experiment can be repeated with solutions of equal concentrations.
3. The impregnation of the filter papers by the solutions. Although unlikely, one solution may be better absorbed by the filter paper due to its certain properties, such as viscosity or ionic charge.
4. The brands of the soaps. As only one brand for each kind of soap is used in this experiment, they may not reflect the whole property of that classification. For example, Protex not necessarily reflects the properties of all soaps in the antibacterial soap industry. The use of a single brand may not be enough to make generalizations about the entire concept of antibacterial soap. On the other hand, Protex is the leading brand in antibacterial soap industry in Turkey, and the concept of antibacterial soap is nearly identical to the brand Protex itself. Similarly, the regular soap and Medical-level antibacterial solution used in this experiment are chosen amongst the most well-known and industry-standard brands. Use of a single brand is useful for limiting the extent of this experiment i.e. not the comparison of different brands of soaps.
5. Only one species of bacterium was used. With other species, different results may have been obtained, for example *Escherichia coli* bacteria may be more resistant to a certain type of soap than *Staphylococcus aureus*. This is not actually an error of the experiment, as the extent of this essay is limited to the effect of different soaps on *Staphylococcus aureus* only. However, by using other bacteria as well, a generalization on which soap is more efficient against bacteria could have been made.
6. The nutritional agar used in this experiment does not mimic the human skin, the medium in which the soaps are optimized to be best against bacteria. The effect of using a Mueller-Hinton nutritional agar is more similar to bacterial reproduction on a meal left in moisture and 37°C temperature for 24 hours. However, the Kirby-Bauer

antibacterial testing method is widely used with Mueller-Hinton nutritional agar, and there are no studies that I could find that makes use of the same method in a basal nutritional agar, which hypothetically better mimics the environment on the skin.

VIII. Conclusion

My research question: "*Is there a significant mean difference among antiseptics used in medical institutions, commercial antibacterial soaps and regular commercial soaps in terms of their bactericidal effects on Staphylococcus aureus in laboratory conditions?*" is answered in the light of the results of my study. There is a significant difference in between their efficiencies against *Staphylococcus aureus* bacterium. Medical-level soap a more efficient bactericidal agent against *Staphylococcus aureus* than antibacterial soap, and antibacterial soap was more efficient than regular soap, as expected. Although the method can be modified for more accurate results, I consider the study successful.

The reason I was moved to do my extended essay on this subject was the discussion on whether antibacterial soaps are really more effective against bacteria than regular soaps. However, the extent of this discussion was too large for my capabilities. So, I decided to limit my study to the antibacterial effects of regular and antibacterial soap on a single bacterium. Although there are studies with larger extends and more accurate methods on this discussion, no study that I have came across to compare the effect of antibacterial soaps and regular soaps to the third variable of medical-level soaps. This is the point my essay differs from the other research on this topic; this has never been done before.

Soap has been traditionally used for cleansing purposes since BC, but today it has differentiated into many different classifications. We have many choices for soap today; the competition between the producers forces the producers to come up with new soaps with new properties. The question do we really need these new properties is still an open question.

IX. Appendices

a. Appendix 1

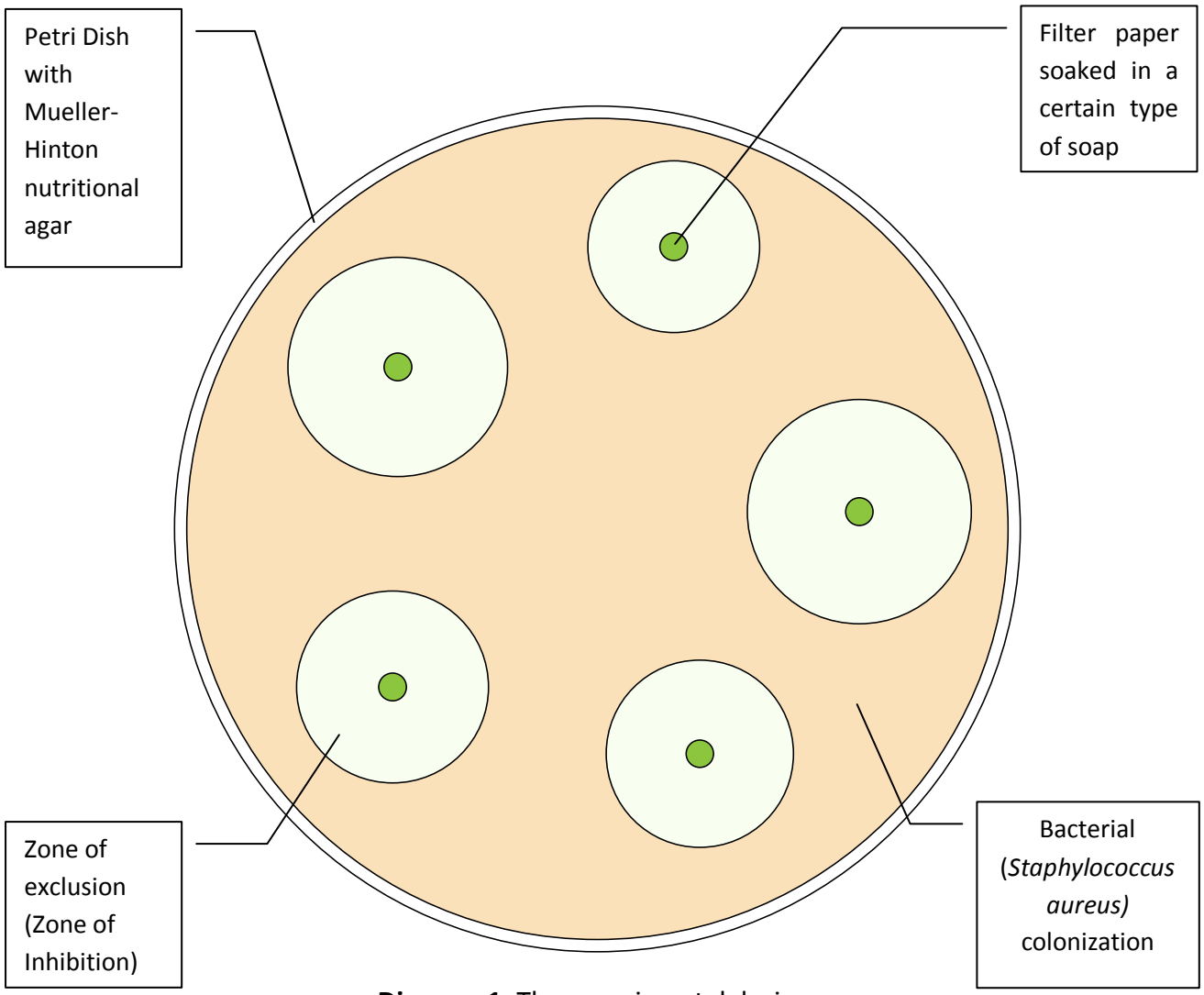


Diagram 1: The experimental design.

b. Appendix 2

Below is information (in quotes) on the McFarland standards, which are used to describe the initial cell density in the nutritional agar. The information is taken from "PML microbiological, Technical Data Sheet #500 Revision 2"[10]

" **McFARLAND STANDARDS**

(...)

PURPOSE:

McFarland standards provide laboratory guidance for the standardization of numbers of bacteria for susceptibility testing or other procedures requiring a standardization of the inoculum. A 0.5 **McFarland** standard is comparable to a bacterial suspension of 10^8 cfu/ml.

PRINCIPLE:

(...) For many types of susceptibility testing, a standard inoculum of bacteria must be used. **McFarland standards** were devised to replace the counting of individual cells and are designed to correspond to approximate cell densities as required by the method of antimicrobial testing.

FORMULAS:

(1)

0.5 McFarland Standard: item no. R6540

Sulfuric Acid, 1%..... 995.00 ml

Barium Chloride, 1%..... 5.00 ml

(...) "

[10] http://www.pmlmicro.com/assets/TDS/500.pdf

c. Appendix 3

Below is information (in quotes) on the Müeller-Hinton Agar, which is the nutritional agar used to as the medium for *Staphylococcus aureus* to reproduce. The information is taken from "Hardy Diagonostics"[11] website

" **(...)**

INTENDED USE

Hardy Diagnostics Mueller Hinton Media is recommended for use in the cultivation of a wide variety of microorganisms. Mueller Hinton Agar is recommended for disk diffusion sensitivity testing of non-fastidious organisms. Mueller Hinton Broth is recommended for preparing suspensions of microorganisms for disk diffusion sensitivity testing.

SUMMARY

Mueller and Hinton developed Mueller Hinton Agar in 1941 to be a protein free medium for isolating pathogenic strains of Neisseria. It was found that Mueller Hinton Agar was useful in identifying sulfonimide-resistant and responsive strains of gonococci. Additionally, in recent times this media has been used in standardized antimicrobial disk susceptibility testing, as described by Bauer, Kirby, et al. Barry and Fay investigated the effects of altering the depth of plated Mueller Hinton Agar on disk diffusion testing, and determined a standardized depth of approximately four millimeters to be sufficient. In 1970 Dewees, et al., studied the effect of storage on Mueller Hinton Agar plates used for antimicrobial disk diffusion zone sizes. Their findings indicated commercially manufactured Mueller Hinton Agar plates were suitable for use in routine susceptibility testing. In addition to the above criteria, Hardy Diagnostics Mueller Hinton Agar meets the standards of performance established by the Clinical Laboratory Standards Institute (CLSI - formerly NCCLS).

Mueller Hinton Media contains beef infusion and casamino acids, and starch. Starch acts as a colloid that protects against toxic material in the medium. Beef infusion and casamino acids are provided as a source of energy and nutrients. Agar is added when a solidifying agent is needed. The levels of tetracycline and sulfonamide inhibitors, thymidine, thymine, magnesium and calcium ions are controlled so as not to interfere with susceptibility testing and to yield good growth.

[11] https://www.hardydiagnostics.com/catalog2/hugo/MuellerHintonMed.htm

The Kirby-Bauer antimicrobial disk diffusion procedure is used with Mueller Hinton Agar plates. It is based on an antimicrobial diffusing through an agar gel, when placed on the agar surface after it has been impregnated onto a filter paper disk. (11,14) Zone diameters established for each antimicrobial determining resistant, intermediate, and sensitive results for pathogenic microorganisms are listed in the Clinical Laboratory Standards Institute (CLSI - formerly NCCLS), document M2-A, Performance Standards for Antimicrobial Disk Susceptibility Tests.(11)

Mueller Hinton Broth is the same formulation, without the added agar. It is used for the cultivation of microorganisms, and for **making dilutions of organisms to be used in the Kirby-Bauer disk diffusion procedure.**

FORMULA

Ingredients per liter of deionised water:*

Acid Hydrolysate of Casein	17.5gm
Beef Extract	2.0gm
Starch	1.5gm

In addition, Mueller Hinton Agar contains:

Agar	17.0gm

Final pH 7.3 +/- 0.1 at 25 degrees C.

* Adjusted and/or supplemented as required to meet performance criteria. (...) **“**

X. Bibliography

The references are written in MLA style.

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