Testing the bactericidal efficiency of an anticeptic, SOW by comparing it with regular soap on hands of hospital staff in Hacettepe University.

Extended Essay (Biology)

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Abbreviations

SOW: Super Oxidized Water

MRSA: Methicillin Resistant *Staphylococcus Aureus*

TSA: Triptic soy agar

TSB: Triptic soy broth

ABSTRACT

In this project, the aim was to test the bactericidal efficiency of an hand antiseptic called SOW, by comparing it to a regular soap. Disinfectants are used to reduce the bacteria on the hands of the hospital staff to diminish the risk of contamination of the bacteria to other patients. Disinfectants in hospitals are tested often because the hospital acquired diseases are seen commonly in Turkey and it is necessary to be hygienic in order to prevent those diseases. If a disinfectant material is not effective, it can cause the bacteria to spread to other patients and it would cause diseases. Soap is a widely used disinfectant and is cheaper than SOW. In order to understand whether SOW is effective or not, soap is used as a comparator. There are several methods for testing the efficiency of a disinfectant. It is necessary to find out which of the testing methods is more suitable to get more accurate results. In this experiment, the surgical hand antisepsis method was chosen because the disinfectant, SOW, was going to be used in hospitals.

In the experiment, the 14 staff who work in Hacettepe University Hospital were chosen. They were asked to wash their hands with regular soap and then they were divided into two groups, seven in each, one using the SOW while the other does not. Both of the groups put their hands into petri dish. After the incubation of the buyyon into petri dishes, the bacterial colonies were counted. By using statistical tests, it was found out that both of the disinfectants were effective. Afterwards, by calculating the percent decreases in colony counts after disinfectant use, the statistical analysis showed that there was not a meaningful difference in the efficiency of both

disinfectants in terms of reducing bacterial colonies. In conclusion, these results suggest that SOW is not a superior disinfectant compared to soap and both can be used in hospitals to reduce the contamination of bacteria.

I. Introduction

Hospital acquired infections are major problems both in developed and resource-poor countries[1]. Infections acquired in hospitals are significant concerns not only because they cause deaths, but also they increase health problems among hospitalized patients[1,2,3]. Hospital acquired infections do occur worldwide and, like in most countries, they lead to serious drawbacks in patient management in Turkish hospitals[4].

Surgical infections, urinary tract infections, respiratory tract infections, catheter infections and septicemias are of the most frequent hospital-acquired infections. The WHO study, and others, have also shown that the highest prevalence of hospital-acquired infections are reported in intensive care units and in acute surgical and orthopaedic wards[1].

Many different measures are being taken under the concept of *infection control policies* to minimize the burden of hospital-acquired infections. Handwashing is recognized as the basic measure for preventing hospital-acquired infections[5]. Alcohol based disinfectants are widely used in hospital setting and generally they are well tolerated. The efficacy of hand rubbing with alcohol based solutions *versus* hand wash with unmedical soaps have been compared in many different studies [6,7]. The efficiency of alcohol-based hand disinfectants has been demonstrated by the scientists in reducing the bacterial count on artificially contaminated hands. In a clinical situation, several studies still show that hand rubbing with alcohol based hand disinfectants is more efficient than with unmedicated soap[5] .The net effect of hand washing with standart unmedical soaps have been found to be superior than some of the alcohol based disinfectants thus their efficacy was debated for some microorganisms[6,7].

Non–alcohol-based products offer some advantages over conventional hand disinfectants: they are non-flammable, safe to store and transport as a concentrate[8].

Being a non alcohol based disinfectant which does have a +1100 mV reduction potential, the Super Oxidized Water (SOW) removes electron from bacteria causing their death. SOW can be used to wash hands, utensils, fresh vegetables for disinfection purposes. Previous tests have proved that it can immediately destroy Methicillin Resistant *Staphylococcus aureus* (MRSA), which is a highly pathogenic microorganism threatening the human health.

Although SOW is a strong sterilizing agent, it does not cause toxic effects on human skin. Therefore, its use is very convenient to sterilize the hands of the staff working in the hospital. This is very crucial to prevent bacterial spreading from the hands to the patients.

SOWs have been investigated as disinfectants for instruments and surfaces in hospitals[9]. SOWs have been tested against bacteria, viruses, fungi, and spores. Freshly generated superoxidized water is rapidly effective (<2 minutes) in achieving a significant reduction of pathogenic microorganisms. However, the bacteria-killing activity of this disinfectant decreased substantially in the presence of organic material (e.g., 5% horse serum). No bacteria or viruses were detected on artificially contaminated medical instruments after a 5-minute exposure to superoxidized water. SOWs have also been recommended for handwashing in medical personnel[9, 11].

Testing of disinfectants periodically to evaluate their efficacy and potency is of great importance. The present investigation is undertaken to compare the net effect of SOW with regular unmedical soap on hand disinfection capacity.

The terms *Sterilization* and *Disinfection* are used throughout the text. *Sterilization* describes a process that destroys or eliminates all forms of microbial life and is carried out in health-care facilities by physical or chemical methods. Steam under pressure, dry heat, Ethylene oxide gas, hydrogen peroxide gas plasma, and liquid chemicals are the principal sterilizing agents used in health-care facilities[12] . Sterilization is intended to convey an absolute meaning; unfortunately, however, some health professionals and the technical and commercial literature refer to “disinfection” as “sterilization” and items as “partially sterile.” When chemicals are used to destroy all forms of microbiologic life, they can be called *chemical sterilants*. These same germicides used for shorter exposure periods also can be part of the disinfection process (i.e., high-level disinfection) . *Disinfection* describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects, such as furnishings, tables, shelves. In health-care settings, objects usually are disinfected by liquid chemicals or wet pasteurization[12].

II. Hypothesis

Many different disinfectants are being used for many different purposes, for example, gluteraldehytes which is an efficient disinfectant; ethylene oxide; which penetrates even inside the instruments.

SOWs have many different advantages than the usual common disinfectants used in the hospital setting such as it is cheaper, fast acting (within 30 seconds), non-toxic, nature friendly.

As my father works in a hospital as a medical staff, I heard about the importance of hand disinfection to avoid hospital acquired infections. As a matter of fact, I have also heard that some other disinfectants with same or superior efficiencies are being implemented as substitutes for alcohol base disinfectants recently. That is why I was interested in whether SOWs- a novel group of disinfectants suggested by many papers in literature[13, 14, 15] are superior in terms of hand disinfection capacity compared with conventional soap.

Since hand washing is reported to be the most important parameter in terms of avoiding hospital-acquired infections, many different alcohol based, non alcohol based disinfectants *versus* regular unmedical soaps have been tested in several settings. As a safer and a cheaper option, SOWs have also been investigated as a means of disinfectants. Marked differences were indicated between the various SOWs tested to date[13]

Although there are many different methods described for testing the disinfection capacity of various disinfectants, the most suitable and rational way to test the effectiveness of surgical hand antiseptics is via “Surgical Hand Antisepsis“ method[12].

The significance of this study is that if SOW is superior to soap, then all the medical staff maybe encouraged to disinfect their hands with this solution instead of soap to protect themselves and their patients from being infected by microbes.

In this study, I aimed to test the hypothesis that the efficacy of SOW was as good as or better than the regular unmedical soap on surgical hand disinfection utilizing “Surgical Hand Antisepsis“ method. In order to prove this hypothesis the protocol described by Kayhan Cağlar[12] was followed. The aforementioned study was conducted at Hacettepe University with Intensive Care Unit staff members to test the hypothesis that potential efficacy of SOW on hand disinfection is greater than regular unmedical hand soap washing.

III. Method Development

There are several methods for testing the efficiency of a hand disinfectant. For example; hygienic hand disinfection and surgical hand disinfection test. In hygienic one; the hands of the subject are contaminated with microorganisms. In the surgical one; the hands are not contaminated, and the efficiency of the disinfectant on eliminating the microorganisms which are naturally living on the skin of the hand is tested. In this experiment, the surgical hand disinfection test was chosen because the aim was to use the tested disinfectant (SOW) in the staff working in hospitals.

In the experiment, the 14 volunteers were chosen. The subjects were first asked to wash their hands with an unmedical soap in order to remove the transient hand flora and foreign particles which might be present on the hands affecting the test results. Afterwards, the stuff were asked to rub their fingers in triptic soy broth (triptic soy broth served as a media to count the microorganisms) to determine the number of microorganisms which naturally colonize in their hands. This was recorded as initial number of colonies.

Then, the 14 people were divided into two groups; the first group put their fingers into petri dish, containing regular soap and water mixture, and rubbed their fingers in it. To neutralize the disinfectant, the subjects were asked to rub their fingers in neutralization buyyon in order to prevent the disinfectant seem more effective more than it really is. Unless a neutralizer is used, disinfection capacity of the solution will last to a timepoint that is unpredictable by yhe experimental procedures leading to biases or false results. (Reference: see appendix B)

The second group put their fingers into petri dish containing SOW instead of soap and repeated the above mentioned procedures. After then, the diluted samples were transferred to petri dishes containing triptic soy broth (TSB). Using the quadrant streak method, the diluted samples were inoculated into agar plates containing triptic soy agar (TSA) solid version of broth (see figure 1 in appendix A). Then the TSA plates were put in 37^{0}C incubator overnight for 24 hours. The colonies which grew after 24 hours of incubation were counted and quantified as actual members. Counting was done as follows: Briefly, the petri dish was divided in each quadrants and, the number of colonies counted in one quadrant was multiplied by four to calculate the total number of bacterial colonies.

IV. Method

MATERIALS

- Super Oxidized Water Solution (mediloks ®) (10 L)
- Distilled water (10 L)
- Triptic soy broth (TSB tubes) (x150)
- Triptic soy agar (TSA plates) (x150)
- Neutralisation broth
- Regular soap
- Board marker for labeling the dishes
- Parafilm
- Regular napkins (x200)
- Bunsen burner (1)
- Small containers to soak paper discs (x5)
- Chronometer
- Test tubes (12 x 75mm)
- Wire loop (x2)
- Ruler
- Incubator

Methods

1- 20 staff members from the department of surgery were randomly chosen.

2- The staff were asked to wash their hands with an unmedical soap for 1 minute as described in surgical antisepsis hand wash guidelines (appendix A)

3- The staff rinsed their hands with tap water for two minutes and dried their hands with a regular napkin.

4- In order to determine the total number of microorganisms before the application of the disinfectant, the staffs were asked to rub their fingers inside the triptic soy broth (TSB) plates for 2 minutes.

5- By this action, the microorganisms on the fingers were mixed with the triptic soy broth.

6- Twenty staff members were then divided into two random groups.

7- In petri dishes, 100 mL of water is mixed with 20 mL of regular soap.

8- The first group (7 persons) put their right hand fingers into the petri dish which had 10 ml of regular soap and water in it and rubbed their fingers for 2 minutes.

9- Afterwards, they were asked to rub their fingers inside the 10 ml neutralization buyyon for 2 minutes.

10- The other 7 persons (second group) were asked to insert their fingers into petri dishes containing 10 ml SOW and rubbed for 2 minutes.

11- They rubbed their fingers for another 2 minutes within petri dishes containing 10 ml neutralisation buyyon.

12- After 1/10 and 1/100 serial dilutions, the mixture was transferred into triptic soy agar (TSA). 1 ml of the sample was taken out from the tube and transferred onto another tube containing 9 ml of TSB for the first dilution. Second dilution was performed via taking out 1 ml of the used original TSB plate and then added on top of a tube containing 99ml of TSB.

13- After these applications, the samples 1/10 and 1/100 times diluted were transferred to TSA plates.

14- Quadrant streak method was applied for the inoculation of the broth onto TSA plates (Appendix 2).

15- The inoculated TSA plates were incubated at 37 ^{0}C for 24 hours.

16- The readings were performed at 24th and colony counts were determined.

17- Colony counts were performed as follows: Plates were divided into four estimated equal squares and the literal microorganism count in each square is identified and then multiplied by four to get the exact number of microorganisms. Plate counts were performed for each plate with or without dilutions.

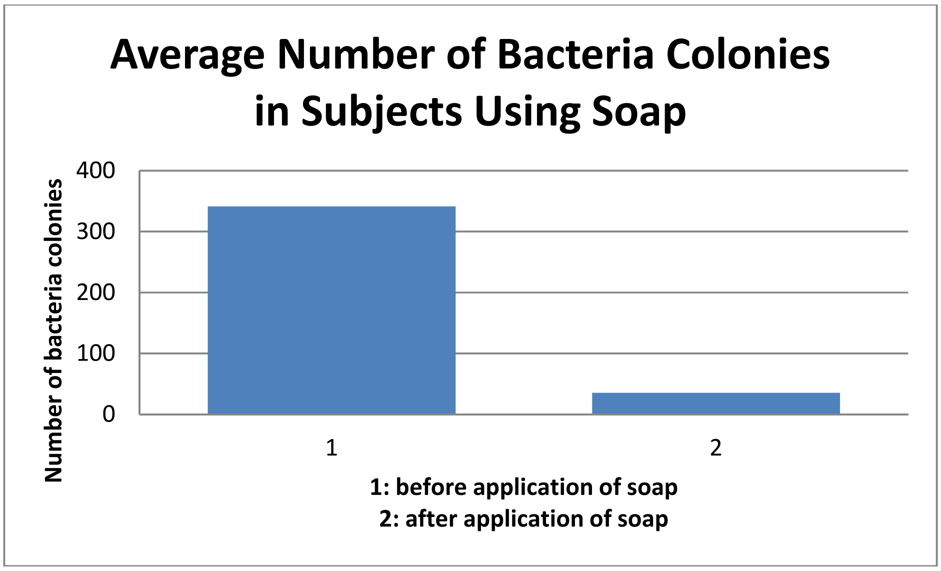
V. Data Collection

Type of disinfectant	Number of subjects	Initial number of bacteria in agar (before putting the hands to soap) ±√n	Final Number of bacteria after putting the hands to soap or SOW ±√n	Duration of experiment (hours) ±1	Temperature of incubation (^{0}C) ±0.5
Soap	**1**	**250 ±√250**	**100 ±10**	**24**	**37.0**
	2	**300 ±√300**	**3 ±√3**	**24**	**37.0**
	3	**70 ±√70**	**20 ±√20**	**24**	**37.0**
	4	**700 ±√700**	**30 ±√30**	**24**	**37.0**
	5	**120 ±√120**	**30 ±√30**	**24**	**37.0**
	6	**450 ±√450**	**25 ±5**	**24**	**37.0**
	7	**500 ±√500**	**40 ±√40**	**24**	**37.0**
SOW	**1**	**40 ±√40**	**35 ±√35**	**24**	**37.0**
	2	**50 ±√50**	**4 ±2**	**24**	**37.0**
	3	**200 ±√200**	**15 ±√15**	**24**	**37.0**
	4	**250 ±√250**	**30 ±√30**	**24**	**37.0**
	5	**500 ±√500**	**2 ±√2**	**24**	**37.0**
	6	**400 ±20**	**30 ±√30**	**24**	**37.0**
	7	**300 ±√300**	**10 ±√10**	**24**	**37.0**

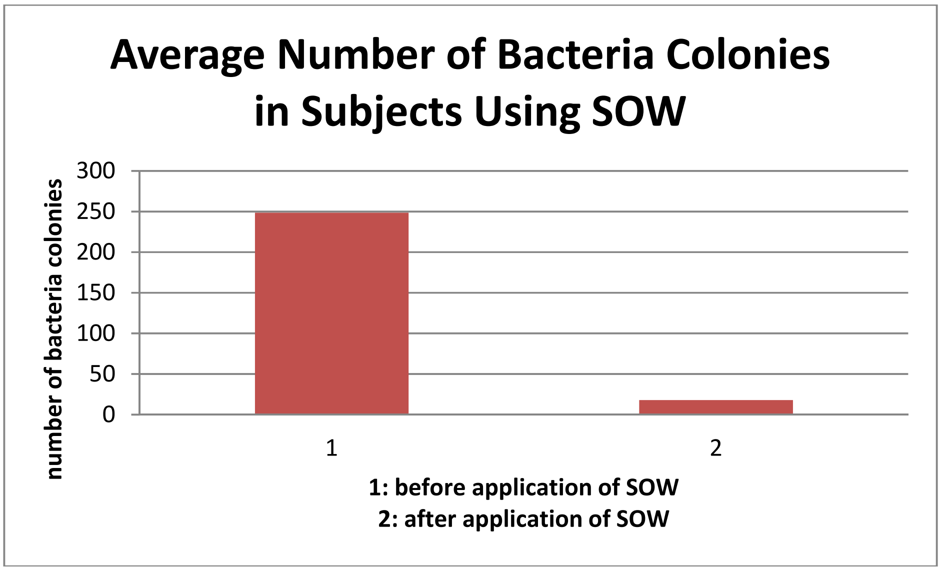
Table 1: Raw data table showing the number of bacterial colonies before and after the application of disinfectants (Soap and SOW)

Since the counts are done directly by eye, uncertainties were expressed as ±√n, where the n expresses the number of bacteria colonies.

BAR GRAPHS THAT INDICATE THE EFFECIENCY OF EACH DISINFECTANTS



Graph 1: Bar graph of the average number of bacterial colonies when the soap is applied to the subjects and comparison of before application and after application of soap.



Graph 2: Bar graph of the average number of bacterial colonies when the SOW is applied to the subjects and comparison of before application and after application of SOW.

VI. Data Analysis

Calculation of Percent decrease

In order to determine whether there is a significant change between the disinfectants used, the final number of bacteria are subtracted from the initial number of bacteria. Afterwards, the percent decrease is determined by using the direct proportional method. The change in the counted bacteria colonies is first calculated in each trial. Then, as the change in the number of bacteria is directly proportional with bacteria number, the percent decrease in bacteria is calculated. For example; If the number of bacteria in initial is 250 and the final is 100; by subtracting 100 from 250, the difference is found as 150.

If it changes 150 in 250 bacteria,

How much it changes in 100 bacteria?

It is calculated as;

$250*x=150*100$

Where x is the percent decrease in bacteria, x is found as 60.

The same calculations are made for all 7 trials of each group.

PERCENT DECREASE IN BACTERIA COLONIES

TABLE 2: Percent decrease in bacteria colonies calculated by initial number of bacteria in agar – final number of bacteria in agar.

	Number of subjects	Percent decrease in number of bacteria colonies (%)
Soap	1	60.0
	2	99.0
	3	71.0
	4	95.7
	5	75.0
	6	94.4
	7	91.7
SOW	1	12.5
	2	92.0
	3	92.5
	4	88.0
	5	99.6
	6	92.5
	7	96.0

Table 2: Percent decrease in bacteria colonies calculated by initial number of bacteria in agar and final number of bacteria, when soap is used and soap is used.

Statistical Analysis

Analysis Table:

	Soap	**SOW**
Mean	**83.8**	**81.8**
Standard Deviation	**15.0**	**30.8**
95% CI	**1.2**	**2.1**
Standard Error	**5,1156**	**11,6418**

Table 3: Data analysis of the percent decrease in the number of bacteria colonies after using the disinfectant.

Student's t test was used to compare the means of two independently collected quantitative data sets. The distribution of the data and number of subjects in each group was similar, so it is chosen as the statistical test appropriate statistical test in this study.[15]

t-test Table:

	Soap	***SOW***
Mean	83,85714286	81,85714286
Variance	226,4761905	933,8095238
number of cases	7	7
t Stat	0,223142401	
P(T<=t) one-tailed	0,415413797	
P(T<=t) two-tailed p	0,830827593	

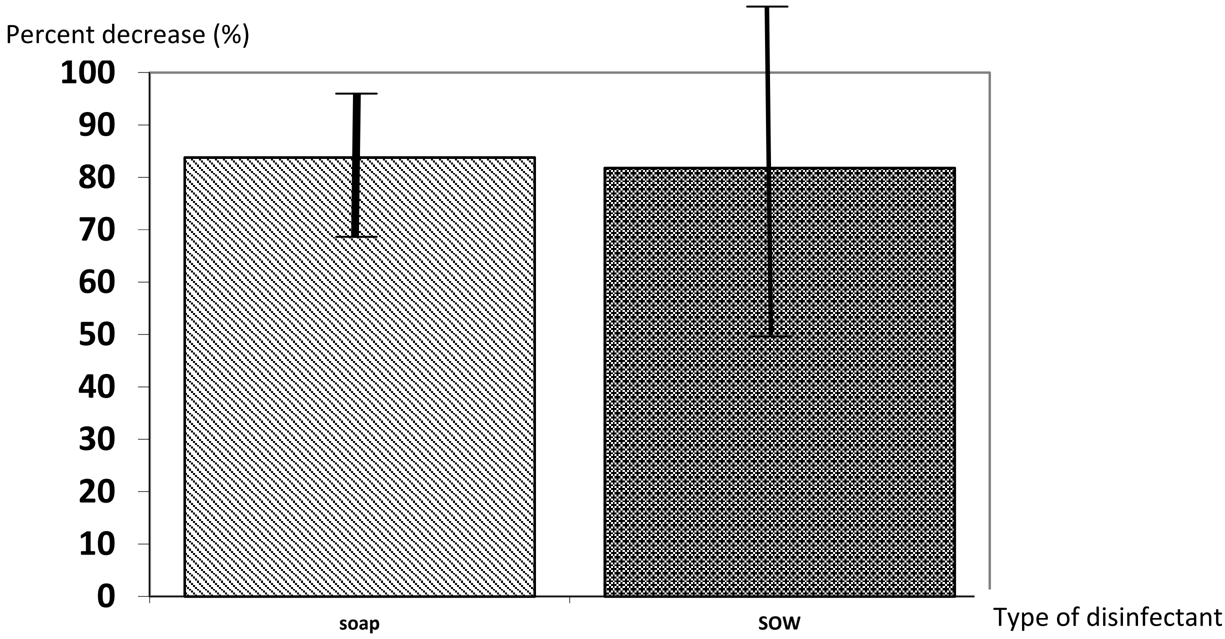
Table 4: t test to compare the change (percent) of bacterial counting before and after application of disinfectant.

Hypothesis of the test:
H_1: There is significant difference between the bactericidal efficiencies of SOW and soap and SOW is superior to soap when used in the hospital staff in Hacettepe University.

H_0: There is no significant difference between the bactericidal efficiencies of SOW and soap used in the hospital staff in Hacettepe University.

Since p value derived from t-test is 0.83, which is too much greater than 0.05, the hypothesis that one disinfectant is superior than the other, is rejected. This means that the two disinfectants have comparable efficiencies in terms of reducing the bacterial colony counts.

GRAPH OF COMPARISON OF THE BACTERIAL EFFICIENCY OF DISINFECTANTS



Graph 3: Bar graph of the comparison of percent decrease of bacteria colony counts before and after the application of disinfectant.

VII. RESULTS

The effect of disinfectant use on colony counts was checked at 24^{th} hour after the application. The raw data of my experiments are shown in table 1. In both groups, application of the disinfectants resulted in a significant decrease in bacterial counts at 24 hour as shown in bar graphs 1 and 2. As there was a significant efficiency of both of the disinfectants, the comparison between them could be made. In order to compare the efficiency of both the disinfectants, the percent decrease among the each trial was calculated. (see table 2). The averages of the percent decrease in both of the disinfectants were calculated. It is found that the average percent decrease in bacteria colonies (before the application minus after the application) for the soap is 83.8 and the SOW is 81.8. When the bar graphs of the average percent decreases were drawn, it is clearly seen that there is no significant difference between them.

Using the percent decreases, the effectiveness of the SOW was tested. The aim of the experiment was to test whether the SOW was an efficient disinfectant. In t-test, p value which is a critical value, which is a level of significal meaning. In the t-test p value should be smaller than 0.05 in order to reject the null hypothesis. According to the t-test which is done to the means of the percent decreases in soap and SOW, it can be seen that the SOW does not have any extra effect. So It does almost the same effect to the bacteria, as the p value is 0.83, which is larger than 0.05.

VIII. Evaluation

The aim of this study was test the efficiency of SOW, comparing to regular unmedical soap using the Surgical Hand Antisepsis method. It was suggested before in many papers that there was a significant difference regarding their disinfection capacity.

My null hypothesis was that there was no significant mean difference between unmedical soap and SOW in terms of their decreasing the number of bacteria colonies. Compatible with this hypothesis, the analyses of the data revealed no significant difference between SOW and unmedical regular soap in terms of reducing the bacterial colony counts. In other words, Soap and SOW were equally effective for controlling the bacterial growth in the hands of the staff.

The hypothesis which was "the efficacy of SOW was as good as or better than the regular unmedical soap on surgical hand disinfection utilizing "Surgical Hand Antisepsis" method. My hypothesis that potential efficacy of SOW on hand disinfection is greater than regular unmedical hand soap washing has not been supported by the result of the first experiments conducted and data analysis of those experiments also indicated that unmedical hand soap was as good as SOW in terms of the capacity for surgical hand disinfection.

So I could interprete my results saying that our null hypothesis was proven by the very first experiments I conducted.

IX. Conclusion:

I have deliberately chosen SOWs because there are many controversial papers in the literature with various outcomes, the efficacy of SOWs being different in terms of surgical hand disinfection. SOW show bactericidal activity against all the microorganisms that grew on baseline plates and yield to reduction in numbers regardless of the microorganism.

My results indicate the fact that although the sample size is not high enough to draw net conclusions, still it is suggestable that the affect of SOW is comparable to unmedical soap.

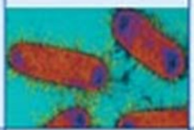
The topic I've chosen for my extended essay was about SOWs mainly because I have read in the literature and in many reports that some of the hospitals in Ankara have started to use SOWs for disinfecting some main laboratories. Having done all the experiments with more staff members and getting more colony counts will definitely be more helpful both in deciding whether to use this disinfectant as a main antiseptic or not.

For now I can still debate that as a safer and a cheaper option, unmedical soap could still be used as an effective hand antiseptic particularly in resource poor countries where the prices are of serious concern. Ongoing search should identify the net effect of SOW on different microorganisms.

Appendices

Appendix A

Phase 2 / Step 2 standards (practical tests) in hand disinfection

European Norm	Application	Test micro-organisms	Conditions/ Exposure time	Requirements
EN 1500	Hygienic hand disinfection / bactericidal effect	*Escherichia coli* 	1 minute or less	The test preparation's efficacy may not be significantly inferior to the reference treatment (2-propanol, 60 % vol)
EN 12791	Surgical hand disinfection / bactericidal effect	Normal skin flora 	5 minutes and less	The test preparation's immediate and 3-hour efficacy may not be significantly inferior to the reference treatment (1-propanol, 60 % vol)

The method used in the experiment was EN12791, a method for hand disinfection.[16]

Appendix B

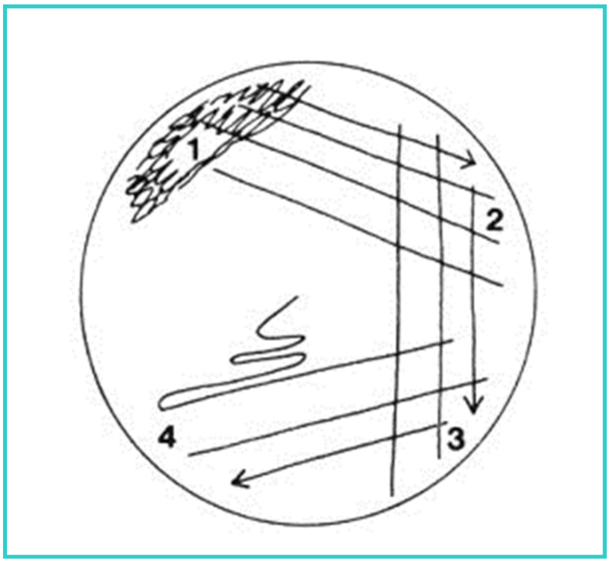
1. **Ingredents of triptic soy agar:**

Peptone from casein 15,0 g/L; Peptone from soymeal 5,0 g/L; NaCl 5,0 g/L; Agar-agar 15,0 g/L.

2. **Ingredents of triptic soy broth:**

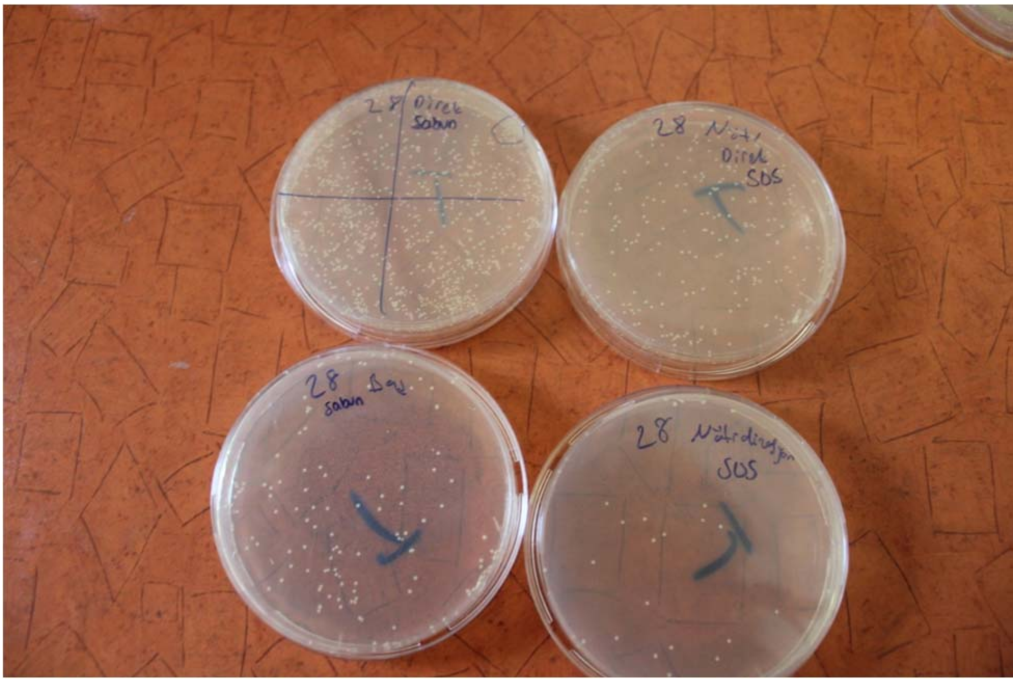
Casein peptone (pancreatic), 17 g/L dipotassium hydrogen phosphate, 2.5 g/L glucose, 2.5 g/L sodium chloride, 5 g/L [17]

Appendix C



Strake plate method which is used to dilute the bacteria in agar.

Appendix D



The bacteria colonies in TSA, soap and sow.

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