Extended Essay

The Effects of Seven Different Soaps on the Growth of Escherichia Coli

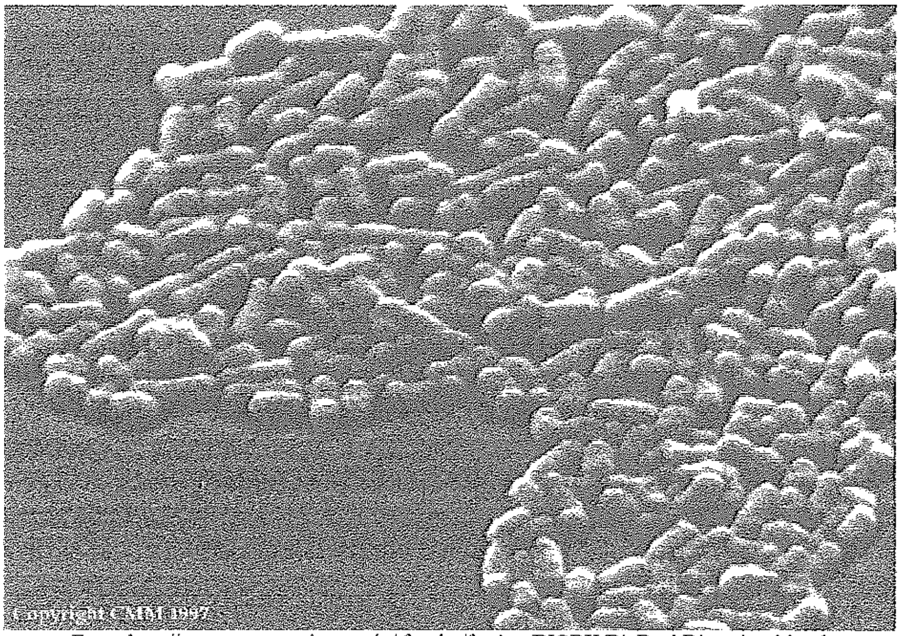

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Table of Contents:

Abstract:

Soap is probably the most known and common disinfectant in this world. However do all types of soap really kill all bacteria present? And is this necessarily a positive aspect? Bacteria were first discovered in the year 1683. Due to this life changing discovery of the two men Grew and Hooke, more research could be done on bacteria and soon disinfectants became involved. The aim of this experiment is to determine the best and the worst out of seven different soaps in their ability to inhibit the growth of Escherichia coli. By knowing this, many food-borne bacterial diseases will be able to be prevented; just by washing your hands with the right soap. It was not surprising that the anti-bacterial soap was the best disinfectant, however after some research the question whether this is a benefit arose again. By doing some research, it was concluded that it is not a benefit to kill all bacteria present on the skin nor elsewhere. The experiment was intended to find the best soap, and it was found.

The Effect of Seven Different Soaps on the Growth of Escherichia coli

Theory: The history of Bacteriology starts in Delft, Holland. Here, a man called Anthony van Leeuwenhoek lived and owned a dry-goods store. However, this was not his passion, almost all his time was devoted to making lenses and therefore making the first microscopes, which opened his eyes to a whole new universe. With the help of the lenses, Leeuwenhoek was the first to discover microbes (a microbe is defined as a "minute life form; a micro organism, especially a bacterium that causes disease. Not in technical use."). In 1676, the discovery of "animalcules" took place. Leeuwenhoek looked at different watery infusions of pepper and ginger; the two English scientists, Grew and Hooke, repeated these observations one year later. In 1683, after sufficient amount of research and magnifications, bacteria (defined as "any of the unicellular, prokaryotic micro organisms of the class Schizomycetes, which very in terms of morphology, oxygen and nutritional requirements and motility, and may be free-living, saprophytic, or pathogenic, the latter causing disease in plants or animals.") were discovered form the white matter collected on teeth. The boom period of microbiology followed, with Weffler, Gaffley, Pfeiffer, Kitasato, Welch and many more. Due to their mistakes and observations made, it was possible for us to know more and more about the bacteria.

One of the newly gained knowledge, and maybe one of the most important subjects in human life, is how to induce the death of bacteria; disinfection. This was an issue of high interest to scientists, because it would mean the prevention of infectious diseases and preservation of foods and other substances from decomposition. Many microbes when treated with heat and chemicals react in a similar way. This means, the cells don't all die at the same time; each individual cell has lesser or greater resistance to the outer factors induced.

Bacteria, however, act very differently to this. Most of them die in the beginning of the period of heat or chemicals put and after, the rate of death falls drastically and continues to fall.

The destruction of bacteria, or sterilization (the word "to sterilize" is defined as "to make free from live bacteria or other micro organisms) can be reached by several methods. Some physical agents include: drying, heat, light or radiation. However, this is not disinfection. Using chemical substances, which are toxic when present in sufficient amount, for the destruction of micro organisms, would be a definition more suitable.

The disinfection of an area can therefore be reached by disinfectants. Many of these, which are sold in supermarkets, are worthless or no better than cheaper substances. Two of the chemical agents are Acids and Alkalis. These work by virtue of the hydrogen or hydroxyl ion due to the concentration of

Footnotes:
1., 2., 3., 5.. Taken from The American Heritage dictionary of the English Language. Pages: 1139, 136, 1763, 81

their solutions. For use on humans (e.g. skin) they are generally too destructive, however they are often used as preservatives. Another group of chemicals often used in disinfection are oxidizing agents, for example, iodine. This liquid chemical is often used on skin due to its penetrating power. Another oxidizing agent is chlorine. It is often used for the purification of water supplies, because it coagulates proteins, this means it inactivates the enzymes within the bacterial cell and therefore destroys it. The most popular or known disinfectant is carbolic acid or also known as phenol. This chemical is taken as a standard with which to compare disinfectants of a similar chemical nature to; the Phenol Coefficient measures the efficiency of all disinfectants. IN 1867, Joseph Lister first used this as a germicide spray in aseptic surgery. With it, he reduced the mortality of postoperative surgery up to 45%.

As more research has been done, the final definition of disinfection can be said to be "measures which kill or inactivate potentially harmful micro organisms, but which do not necessarily kill all the micro organisms present; it has little or no effect on endospores" (*Introduction to Bacteria*). The difference between disinfectants and antiseptics is that antiseptics (defined as "of or relating to, or producing antisepsis"; antisepsis is defined as the "Destruction of disease causing micro organisms to prevent infection.") can the applied safely to skin and other tissues. However, the general properties apply equally to both. The reactivity of both is affected by dilution, temperature, pH, and presence of hard organic matter, soaps and detergents. The general definition of soap is "a cleansing agent, manufactured in bars, granules, flakes, or liquid form, made from a mixture of the sodium salts of various fatty acids of natural oils and fats."

Escherichia Coli comes from the family Enterobacteriaceae. The word Escherichia means Genus and the word Coli means Species. Most kinds of these bacteria are necessary for our development and our body's functions; they live in our colon (intestines). These bacteria provide us with necessary vitamins such as Vitamin K. However, some strains of Escherichia Coli, like E. coli O157:H7, can cause loss of blood in our body. The reason why I chose to do this experiment is because, it is known that bacteria can cause harmful diseases. For example, Escherichia Coli is the main food-borne bacteria present mostly in raw meat; it can cause poisonings of various kinds in humans. With this experiment using different soaps (see specific research question below), I will be able to determine the best soap to prevent Escherichia Coli on the skin.

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<u>Aim:</u> To investigate the effect of seven different soaps (expensive body wash with perfume, expensive soap bar with perfume, cheap soap bar with perfume, soap bar without perfume, cheap body wash with perfume, pH neutral soap without perfume, anti-bacterial soap) on the growth of Escherichia coli.

Hypothesis: I predict the soaps acting best as an antiseptic or disinfectant to Escherichia Coli will be the anti-bacterial soap and the non perfume containing soaps. The soaps acting the worst as an antiseptic or disinfectant to Escherichia Coli will be the perfumated soaps. The remaining soaps will be somewhere "in the middle", meaning not as effective as the best disinfectants however not as poor as the worst disinfectants.

This prediction is based on experiments done before, which have had the conclusion that anti-bacterial soap kills 0.2% more bacteria than normal soap. It is also based on the fact that anti-bacterial soaps often contain chemicals such as triclosan, which prevent the bacterium to form a cell wall along with preventing its normal functions. "Normal" soaps only have the effect of preventing the bacteria's normal functions.

Method:

Step 1 – Making agar Petri dishes

Materials:
-test tube rack
-Bunsen Burner
-bench mat
-eight sterile Petri dishes
-two sealed beakers with sterile nutrient agar
-nine beakers
-glove

Procedure: All materials are gathered around a working space. The two beakers with sterile nutrient agar are placed in the microwave for approximately ten minutes. They are removed using the glove. The lids are removed and the neck of the beaker is held in the blue flame of the Bunsen burner for three seconds, to sterilize the beaker. Simultaneously, the lid of the sterile Petri dish is lifted and the melted agar (approximately 20ml) is poured into it. The lid is only held open slightly, in order to protect it from contamination of airborne bacteria. The closed Petri dish is rotated in order to have the agar cover the bottom of the dish equally. Now the Petri dish is not moved anymore for approximately fifteen minutes, until the agar has solidified itself. In order to prevent contamination of airborne bacteria, sterile techniques are used. This includes leaving the Bunsen burner on throughout the experiment and working relatively close to it. Safety goggles should be worn as well as protective clothing. This procedure is repeated with the remaining Petri dishes and nutrient agar.

Step 2 – Adding Escherichia Coli to sterile Petri dishes

Materials:
-nine sterile nutrient agar plates
-one dish with a lawn of Escherichia Coli (from University of Geneva)
-Sterile spreader glass
-80% ethanol
-beaker
-Bunsen burner

-nine sterile test tubes
-9cm³ of distilled water
-nine sterile syringes
-test tube rack
-bench mat
-inoculating loop

Procedure: All materials are gathered and set up. The nine test tubes are placed in the test tube rack. A syringe is taken and 1cm³ of distilled water is drawn with it and placed into the test tube (avoid contact with walls). An inoculating loop in taken and held into the blue flame of the Bunsen burner for three seconds. The inoculating loop has to be allowed to cool down for seventeen seconds at least, to avoid the killing of the Escherichia Coli. Now it is scraped across the diameter of the Petri dish, containing a grown culture of Escherıchia Coli, twice. This is then added to the test tube with the distilled water and it is shaken to ensure the complete distribution of the bacteria. Now a little bit of the 80% ethanol is poured into a beaker, which is placed as far away as possible from the Bunsen burner to avoid accidents. The glass rod is dipped into the ethanol, when everything is covered it is removed and passed through the blue flame. The glass rod is allowed to cool down for seventeen seconds. The Escherichia Coli containing test tube is poured into the sterile Petri dish and the mixture is spread out with the glass rod to ensure equal growth. This is repeated with the remaining eight test tubes and Petri dishes.
This technique is called making a "lawn". This has to be used in order to have a controlled amount of Escherichia Coli in each Petri dish.

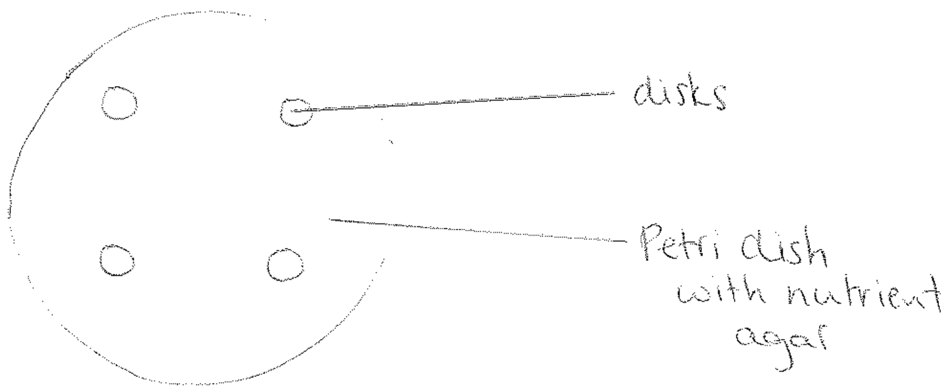
Step 3 – Adding the different soaps

Materials:
-roll of filter paper
-hole puncher
-80% ethanol in a beaker
-cotton swab
-tweezers
-eight beakers with 20cm³ of distilled water
-sterile spoons
-plastic gloves
-Bunsen burner
-nine Petri dishes with Escherichia Coli lawn
-bench mat

Procedure: The sterile roll of filter paper is taken and placed beside the hole puncher, the parts of the hole puncher coming in contact with the paper are sterilized with the cotton swab dipped into 80% ethanol solution. Now the roll of sterile filter paper is passed through the hole puncher and thirty-two sterile filter paper disks are made and placed in another sterile empty Petri dish, a sterile lid is placed on top of it to avoid contamination. Now the tweezers are taken and held into the blue flame of the Bunsen burner for three seconds, while the tweezers are cooling down the soap solutions are made. A beaker with 20cm³ of distilled water is taken, two grams of soap is added to this (by subtracting initial mass from the final mass; answer has to be equal to two).

The mixture is stirred with a sterile spoon until solution is completely aqueous/liquid. This is done with seven beakers; the eighth one will only contain distilled water, to act as a control. Now one sterile paper disk is picked up the sterile tweezers. It is dipped into one soap solution and placed on the corresponding labelled Petri dish. This is repeated three more times; the disks should be spaced out equally on the Petri dish. The disk should be gently pressed down to ensure the contact with the nutrient agar. This is repeated with all the remaining soaps and the distilled water. Each time the tweezers are sterilized to ensure sterile conditions. The Petri dishes are now placed into the incubator at 37°C for 24 hours. Results are recorded.

Diagram of Petri Dish, showing the placement of the disks:



Variables:

Dependent Variable: number of colonies grown, lawn present or absent

Independent Variable: types of soap on disks

Controlled Variable: amount of Escherichia Coli, amount of distilled water, amount of nutrient agar, number of hours in the incubator, number of disks, amount of soap on disks

Results:

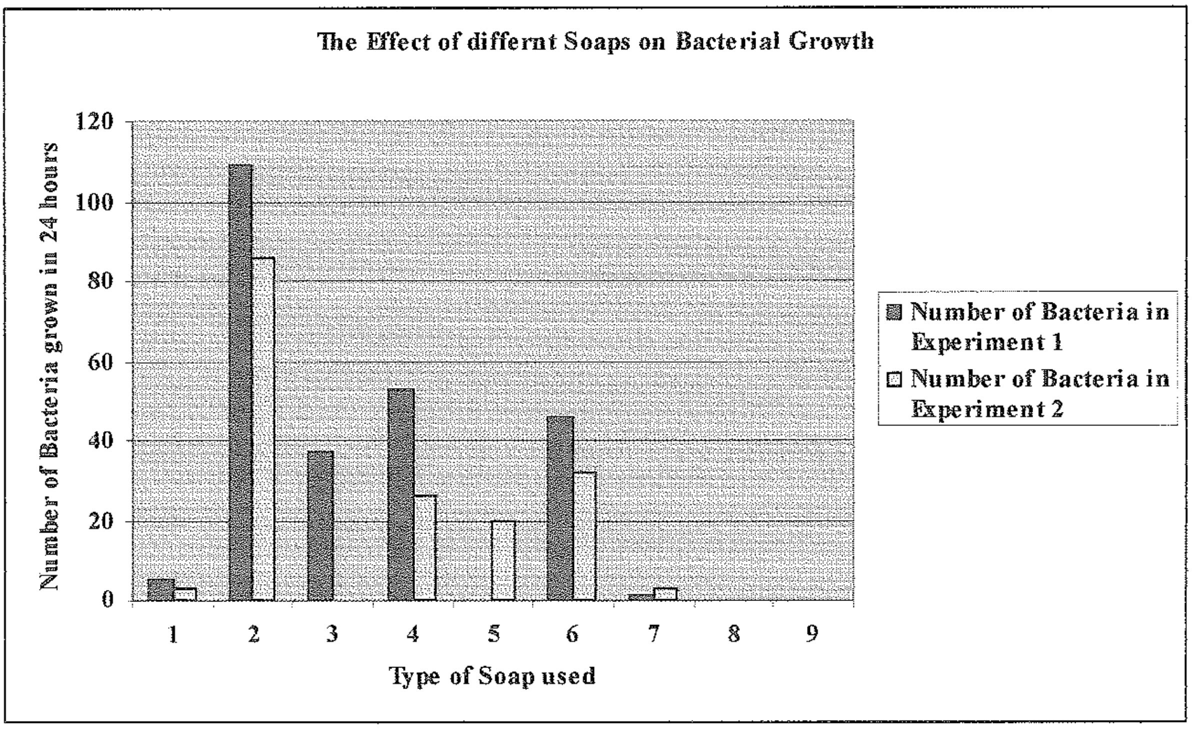
Table 1:

Type of Soap Used	Number of colonies of Bacteria in Experiment 1	Number of colonies of Bacteria in Experiment 2	Observations
Expensive Body Wash with perfume	5	3	Not very many colonies of E coli could be found, however a lawn of E. coli was present in the

			middle of the dish
Cheap Body Wash with perfume	109	86	Also a lawn present, right next to the disks; no "clear" circle around plates
Expensive Soap Bar with perfume	37	Contaminated with unknown bacteria (no valid result)	Very clear gathering of bacteria in the centre of Petri dish
Cheap Soap Bar with perfume	53	26	No "clear" circle around disks; in Experiment 2, more a lawn than in Experiment 1
Soap Bar without perfume	Contaminated with unknown bacteria (no valid result)	20	Clear colonies of E. Coli in centre of Petri dish; two bacteria on the very side of Petri dish
pH neutral soap bar without perfume	46	32	Clear circle of 1cm diameter around the disks; slight lawn
Anti-bacterial soap	1	3	No lawn present.
Just distilled water (control)	0	Contaminated by unknown bacteria (no valid result)	Lawn present in first experiment.
No disks (control)	0	0	

Data Analysis:

Graph 3:



Legend: 1= Expensive Body Wash with perfume
2= Cheap Body Wash with perfume
3= Expensive Soap Bar with perfume
4= Cheap Soap Bar with perfume
5= Soap Bar without perfume
6= pH neutral soap bar without perfume
7= Anti-bacterial soap
8= Just distilled water (control)
9= No disks (control)

Data Analysis:

Results have been recorded in Table 1 (page 6 and page 7) and graph 3 (page 7).

From the numbers obtained, it can be concluded that the most effective soap, in inhibiting growth of Escherichia Coli, is as predicted the anti-bacterial soap bar without perfume. With this particular soap, in experiment one, only one colony of E. coli grew after twenty-four hours and in experiment two, only three colonies of E. coli grew after twenty-four hours. Even further, there was no indication of a lawn on the nutrient agar. The second most effective soap to inhibit growth of Escherichia Coli, in these two experiments was the expensive body wash with perfume. It only had five and three colonies grown in experiment one and experiment two. Even though there was a clear lawn present, this still does not exceed the amount of bacteria which grew in the presence of the other soaps. It is possible to conclude this, because the lawn was only present in the very middle of the dish, so it was approximately one centimetre from each disk. This proves the "disinfecting" ability of the body wash. Third on a list from most effective to least effective is the soap bar without perfume. As seen in table 1, there was no valid result for experiment one, however the number of colonies in experiment two (twenty colonies) and the fact that they grew in the centre of the dish enable us to see rank this soap at this level. The expensive soap bar with perfume and the cheap soap bar with perfume are relatively equal in effectiveness. Both grew many colonies and both grew a lawn, which showed no clear surrounding to the disks. However it does seem that the expensive soap bar with perfume had its colonies slightly more centred than the cheap soap bar with perfume, therefore it can be concluded that this one is slightly more effective in inhibiting growth of Escherichia Coli. By far the worst (least effective) inhibitor of the growth of Escherichia Coli was the cheap body wash with perfume. It grew 109 colonies in experiment one and 86 colonies in experiment two. In addition to this a lawn of bacteria was present which showed no clear surrounding of the disks..

As a final interpretation of the results the list from most effective to least effective soaps in inhibiting growth of Escherichia Coli is: Anti-bacterial soap, expensive body wash with perfume, soap bar without perfume, pH neutral soap bar without perfume, expensive soap bar with perfume, cheap soap bar with perfume, cheap body wash with perfume.

As seen in table 1, colonies of Escherichia Coli have grown on almost each Petri dish. This is not something expected, because the simple strand of bacteria used grows in a lawn on nutrient agar. An explanation for this occurrence is the diffusion of soap. When substances (such as soap) move from an area of high concentration to an area of low concentration, diffusion is taking place. In this way, the soap went all over the nutrient agar, letting the bacteria build a resistance. The bacteria which grew resistant to the soap used, appeared as colonies. Therefore, if a lawn was present throughout the whole of the agar, leaving no space around the disks in the Petri dish, it means that the soap used had no effect at all. These facts support the conclusion reached.

Evaluation: The hypothesis stated on page three, is only proven to be correct partially by the results obtained. It is correct that the anti-bacterial soap has been the best inhibitor of Escherichia Coli compared to all the other soaps used. However, the second part of the hypothesis ("The soaps acting the worst as an antiseptic or disinfectant to Escherichia Coli will be the perfumated soaps") has not been proven as being correct. In fact the results obtained do not show a great difference between perfumated and non perfumated soaps, even though they do seem to show that expensive soaps tend to be better inhibitors of bacterial than cheap soaps.

One of the most interesting and most discussed comparisons done in this experiment was the comparison of anti-bacterial soap to different kinds of not anti-bacterial soap. Even though, the anti-bacterial soap was the most effective, it did not make a great difference as compared to the expensive perfumated body wash (one and three colonies compared to five and three colonies). In fact all soaps are antibacterial. A study done in the early nineties has come to the conclusion that normal soap kills approximately 99.4 % of the bacteria on your skin. This is only difference of 0.2% when compared to anti-bacterial soap (kills approximately 99.6% of all bacteria). This increase in effectiveness comes from special antibacterial chemicals (such as tricolsan; see "ingredients" on page 7) which are contained in the soaps. Tricolsan, for example, works in such a way, to disrupt the normal functions (such as the oxygen uptake, food making etc.) and to prevent the growth of a normal cell wall in Escherichia coli or other bacteria and therefore to make them ineffective. Therefore, this means when different anti-bacterial soaps are compared to each other, what really is being compared is the different amounts and different chemicals added to the particular soap.

A question arises when looking at this comparison between anti-bacterial and normal soap. A logical thinking would probably result in saying "Well anti-bacterial soap does not increase greatly in effectiveness but it is an improvement so it is better right?" However, this is not true, due to two essential problems: the amount of good bacterial living on your skin and bacterial resistance. The good bacteria mentioned lives on the surface of the skin and helps to fight off other harmful bacteria. Antibacterial chemicals do not make a difference between "good" and "bad" bacteria, these chemicals just kill them all. Therefore by using anti-bacterial soaps the chances of becoming sick increase. The second problem mentioned is bacterial resistance. As mentioned before, anti-bacterial soap kills 99.6% of all bacteria on your skin, logically this leaves 0.4% of bacteria on your skin, which have not been killed. From the knowledge of "the fittest survive" the 99.6% bacterial killed first are the weakest leaving the 0.4% to be the strongest bacteria. These use the new space to spread out and multiply. The next time the bacteria are confronted with the anti-bacterial chemical again, the weakest are killed and the strongest survive and reproduce. This is a cycle which continues until the bacteria are so strong that the anti-bacterial chemical cannot kill them anymore; therefore bacterial resistance.

As seen in the results recorded in table 1, the colonies formed were counted. It is not typical for Escherichia coli to behave this way, however a professor of bacteriology from the University of Geneva suggested that even here, a certain resistance towards the soap used could have been established, because the Petri dishes were incubated for a long period of time.

The bacterial resistance is not necessarily a great threat to everyone, the only place where it might become dangerous is in hospitals. Healthy people do not really need the extra 0.2% protection that anti-bacterial soap gives them, however sick people do. When drugs can no longer be used to kill these extra germs, there is a problem.

Even economic reasons exist which suggest not to use anti-bacterial soap. A professor at the National Institutes of Health, Meade, says: "There is a really, really, really slight difference in effectiveness. If you scrub your hands vigorously for 25 seconds under warm water, you will eliminate 99.4% of the bacteria present. With antibacterial soap, you raise it to 99.6 percent."

As seen from the results in table 1 and graph 3, it does not matter whether soap has perfume added or not, relative to the effect on Escherichia Coli and bacteria in general. This proves the hypothesis stated on page three to be partially wrong. Thoughts may be mislead because magazines (such as "seventeen") suggest not to used perfumated soap because "fragrance ingredients are still major causes of allergic contact dermatitis" *(seventeen; see bibliography)*. However, this fact has nothing to do with the anti-bacterial strength of the soap. The ingredients in perfumated and non-perfumated soaps are generally the same except the fragrance chemicals present. Therefore there cannot be any significant difference been soaps with perfume or without perfume in a bacterial point of view.

As stated in the evaluation, there is no great difference between the effectiveness of the soaps on Escherichia Coli and other bacteria. However, this experiment was important and interesting to perform in order to find this and see the consequences of, for example, using anti-bacterial soap. The reason why Escherichia Coli was chosen is because it is one of the main food-borne bacteria present in our present society. It is present in especially in raw meat. In order to protect ourselves and our children we have to know what to do; according to the results obtained and the research made, I would suggest to wash the skin with medium temperature water (approximately 30°C) and to use normal, non perfumated soap. This is to ensure the health of yourself and others.

The method used had quite a few contaminations; however this is most probably due to human error and a misuse of sterile techniques. To prevent this, the experiment should be carried out in a sterile, vacuum room. This would inhibit any other bacterial growth. Also, new instruments/materials should be used to prevent contamination. By repeating the experiment several times, accurate results are more likely to occur. Instead of using a hole puncher to cut out the disks, all ready cut, sterile disks should be used. Due to the diffusion of soap into the nutrient agar, the lawn of bacteria should not be made by a solution, but by direct contact with the bacteria onto the nutrient agar. This prevents the spreading of the soap and would lead to more accurate results. Apart from these changes, and human errors, the method used is excellent for basic experiments on bacteria and disinfectants.

Experiments, as the one preformed, are important in modern society in order to prevent diseases from spreading. It is important to look at the different soaps, study the background and find out whether money can really buy us health, and whether it is really better to kill all than only some. These important questions have been answered. However, this subject deserves a lot more study in detail to protect us and our children.

Appendix:

Ingredients of Soaps:

Expensive Body wash with perfume:	Aqua, Sodium Laureth Sulfate, Glycerin, Cocamidopropyl Betaine, Cocamide DEA, Coco-Glucoside, PEG-40 Hydrogenated Castor Oil, Phenoxyethanol, Sodium Benzoate, Parfum, Citric Acid, Carica papaya, PEG-55 Propylene Glycol Oleate, Propylene Glycol, Benzophenone-4, Disodium EDTA, Caramel, Cl 15510, Cl 17200
Cheap Body Wash with perfume:	Sodium laureth sulphate, purified water, cocamide DEA, propylene glycol, orange extract, fragrance, orange oil, methylparaben, propylparaben
pH neutral soap bar:	Corn Starch (Zea Mays), Potassium Lauryl Sulfate, Sodium Lauryl Sulfate, Cetearyl Alcohol, Water (Aqua), Stearic Acid, Disodium Lauryl Sulfosuccinate, Dead Sea Salt (Maris Sal), Fragrance (Parfum), titanium Dioxide, Phosphoric Acid
Soap bar without perfume:	Sodium Tallowate, Sodium Cocoate or Palm Kernelate, Water, Glycerin or Sorbitol, Tinaium Dioxide, Sodium Chloride, Tetrasodium Etidronate, Pentasodium Pentetate
Soap bar with perfume:	Sodium Tallowate, Sodium Cocoate or Palm Kernelate, Water, Glycerin or Sorbitol, Tinaium Dioxide, Sodium Chloride, Tetrasodium Etidronate, Pentasodium Pentetate, fragrance
Anti-bacterial Soap bar:	triclosan 0.2%, sodium Tallowate, Sodium Cocoyl Isethionate, Sodium Cocoate and/or Sodium Laurate, Water, Sodium Isethionate, Stearic Acid, Coconut Acid and/or Lauric Acid, Sunflower Seed Oil, Wheatgermamidopropyl Dimethylamine Hydrolyzed Wheat Protein, Titanium Dioxide, Sodium Chloride, Disodium Phosphate, Tetrasodium EDTA, Trisodium Etidronate, BHT