Determining Factors that Make Various Hand Cleansers More Viable for Spore Removal

by

Emma Griffiths

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Author .....
Department of Materials Science and Engineering
May 7, 2021

Certified by .....
Michael Tarkanian
Senior Lecturer of Materials Science and Engineering
Thesis Supervisor

Certified by .....
Christopher J Di Perna
Technical Instructor of Materials Science and Engineering
Thesis Supervisor

Accepted by .....
Juejun Hu
Associate Professor of Materials Science and Engineering
Chair, DMSE Undergraduate Committee

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Abstract

The seemingly simple act of soap and water hand washing is not possible in all communities throughout the world since millions lack consistent access to clean water. As a result, deadly diarrheal and respiratory illnesses can be more easily spread throughout these communities. Many of these illnesses come from spore forming pathogens. Spores have a very robust structure that makes them difficult to kill. Alcohol-based hand sanitizers (ABHS) are often used as a solution to waterless hand washing, however, ABHS are unable to kill spores. To develop a water-minimal cleanser more suitable, factors that make a cleanser more effective for spore removal need to be determined. Various hand cleanser compositions and textures were tested to determine to what degree the cleanser composition contributes to spore removal versus to what degree the scrubbing and rinsing step contributes to spore removal. Hand cleansers studied included Dove bar soap, Purell, Betadine, Purell coupled with pumice, and wood ash paste. Zone of Inhibition tests and simulated hand wash experiments were performed to determine how hand wash composition and texture impact spore removal effectiveness. No cleanser composition alone was effective in killing the bacteria post growth. Cleansers that acted more as a scrub, such as the Purell with pumice, were the most effective in removing the bacterial spores in a simulated hand wash. In the future, waterless hand cleansers can be further optimized to assist in spore removal. Wet wipes with embedded particulate may be a good solution. The corresponding waste, cost, ingredients, ability to locally manufacture, ease of use, and more will all need to be considered before a final hand cleanser recommendation is determined.

Thesis Supervisor: Michael Tarkanian
Title: Senior Lecturer of Materials Science and Engineering

Thesis Supervisor: Christopher J Di Perna
Title: Technical Instructor of Materials Science and Engineering

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Chapter 1

Introduction

1.1 Motivation

Soap and water handwashing is an important practice when it comes to protecting oneself and others against contracting and spreading pathogens. However, for many across the world this seemingly simple act is not possible due to the fact that it takes gallons of water to wash one's hands throughout the course of the day. Staggeringly, there are an estimated 780 million people worldwide who do not have access to clean water for drinking and basic sanitation[1]. As a result, an estimated 801,000 children perish from diarrhea and dehydration-related diseases each year, and 88% of these deaths are caused by unsafe drinking water, inadequate availability of water for hygiene, and lack of access to sanitation[1]. To combat this, hand sanitizers, such as alcohol-based sanitizers (ABHS), seem like a great first step in developing a waterless hand cleanser. ABHS works well against many pathogens and does not result in increased pathogen resistance long term. However, unlike soap and water washing, ABHS is not successful against the spore forming pathogens that cause these diarrheal and respiratory infections, such as *Cryptosporidium*[2] and *Clostridium difficile*[3]. The alcohol (hydroxyl -OH) in ABHS kills bacteria by rupturing the cell membranes, thus allowing the inside of the cell to spill out. Because the ABHS is never rinsed from the hands, its effectiveness is limited to the degree it is able to lyse the bacteria. Because spore forming bacteria have numerous, robust protective layers (and not simply

a single lipid bilayer), ABHS is not effective in penetrating their membranes. The development of a new waterless (or water minimal) hand cleaning method capable of removing or killing these pathogens is critical in preventing deaths caused by diarrhea and dehydration-related diseases.

Better water-minimal hand washing methods can be produced when it is better understood whether soap's effectiveness is primarily due to its composition or the scrubbing and rinsing step that corresponds with soap and water washing. If the scrubbing and rinsing act significantly contributes to soap's success, then adding a particulate (such as pumice, salt or sand) to a waterless sanitizer and introducing a brief water rinsing step could potentially be as effective as soap and water washing (while still using less water). Developing a new sanitizer recipe with an ingredient such as povidone iodine (one that has shown to have potential promise regarding killing pathogenic spores) may also prove successful. These theories are tested in this experiment. Successful water-minimal hand washing methods will be ones that are more effective at removing or killing these spore forming pathogens.

1.2 Background

1.2.1 Soap and Water Handwashing

Soap and water handwashing has been shown to be effective against numerous pathogens. The molecular structure of soap is tailored to allow easy pathogen removal. Soap is a natural surfactant composed of a polar, hydrophilic head on one side and a hydrophobic hydrocarbon tail on the other side, as shown below in Figure 1-1a. The dual nature of soap molecules allows them to form micelles around dust, oils, germs, and pathogen fragments as shown in Figure 1-1b. The hydrophilic head and hydrophobic tail structure allows the soap molecules to wedge into and disrupt fat membranes of pathogens, rendering them inactive as shown in Figure 1-1c. Coupled with a scrubbing and rinsing step, the micelle formation makes it easy to wash away contaminants because the micelles are readily carried away by the water and take along anything trapped inside

them. It is important to note that the scrubbing and rinsing acts that correspond with soap and water washing are not trivial. In fact, these actions also contribute to soap's bacteria removal effectiveness. It is not well understood in literature if the scrubbing and rinsing act alone is sufficient in removing spores or if the combination with the soap composition is necessary. Both of these cases will be compared in this experiment.

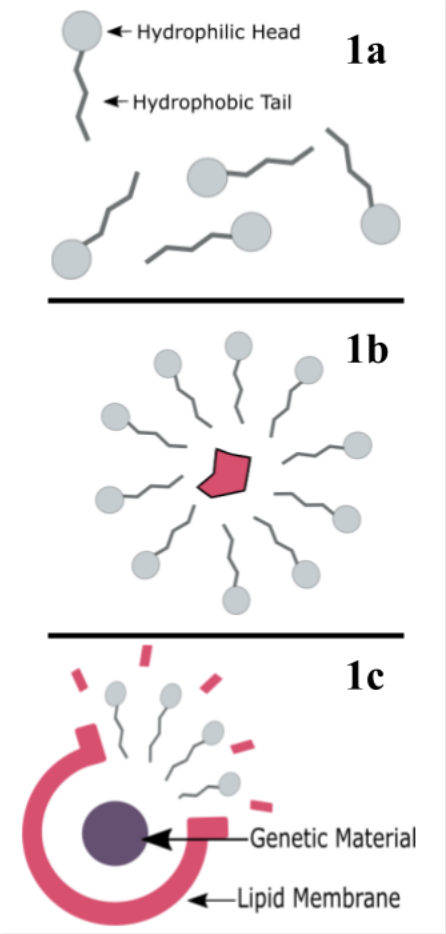

Figure 1-1: 1a. Soap molecules made up of a hydrophilic head and a hydrophobic tail. 1b. Soap molecules forming a micelle. 1c. Soap molecules disrupt the pathogen lipid membrane and pry it apart.

1.2.2 Sporicidal Compounds

There are various substances that can effectively kill bacterial spores without a scrubbing and washing step. However, many of them are far from safe to use on hands. Even just having cracked, dry skin, and hands lacking the natural "resident flora" can reduce one's resistance to pathogens. Some sporicidal compounds include formaldehyde, chlorine-releasing agents, peroxygens, and more. There is one candidate in particular that stands out because it is nontoxic and able to be used as a wash: iodine based compounds.

Iodine concentration and pH both impact spore removal effectiveness[4]. At neutral and acidic pH, diatomic iodine is highly active and hypoiodous acid also has a smaller contribution. At alkaline pH hypoiodide ions form, which has only slight activity, and the inactive iodate, iodide, and triiodide ions. A major problem with iodine itself, however, is that it is toxic and also stains fabric, tissues, and skin.

A solution to this problem is to use iodine in iodophor form rather than simply in an aqueous solution. An iodophor is made by complexing iodine with a solubilizing agent, such as a water-soluble polymer or a surfactant. One of the most common examples of this is povidone, which then can form povidone-iodine. This active ingredient is used in various over the counter antiseptics such as Betadine.

The iodophors consist of a loose complex of elemental iodine solubilized by carriers of neutral polymers and polyethylene glycols[4]. This increases solubility while allowing for the slow release of iodine over time. Depending on desired use, the concentration of free iodine in an iodophor can be altered. The concentration of free iodine is directly related to the overall bactericidal activity[4]. For example, iodophors intended for disinfectants will contain much more free iodine than ones intended for antiseptics. In many iodophors, the iodine forms micellar aggregates inside a nonionic surfactant carrier. Research shows that iodine based compounds can penetrate the cell wall of microorganisms quickly, disrupt the proteins, nucleic acid structure, and synthesis, thus killing the microorganism[4]. Iodophors at high concentrations may be sporicidal

over a wide pH range. They also do not stain and are nontoxic. These factors make iodophors a potential option for a water minimal hand cleanser.

1.2.3 Spore Forming Pathogens

A lot of harmful bacteria are able to form into spores when environmental conditions become unfavorable. These dormant spores are able to outwait the unfavorable conditions and reform into the bacteria once the conditions improve. It is important to understand the structure and lifecycle of spore forming bacteria to understand when the bacteria can most easily be killed and when simply removal is sufficient.

The specific bacteria that cause severe and deadly diarrheal infections include *Cryptosporidium*, norovirus, and *C. difficile*. Spore forming bacteria follow a life cycle and transition between phases depending on environmental conditions[5]. A *Bacillus* spore forming life cycle is shown below in Figure 1-2. The spore-forming *Bacillus* life cycle has three main phases: vegetative growth, sporulation, and germination. The bacteria utilizes numerous signaling pathways to sample the environment and transmit nutritional and growth rate information to make informed decisions about when to transition from one phase to another.

The vegetative growth phase occurs when nutrients are available. Normally, the cell undergoes binary symmetric fission and chromosomal replication. Cleavage often separates the two sister cells. However, the *Bacillus* species will occasionally form long chains linked together through binary fission instead. This is likely due to variations in nutrient availability. An example *Bacillus* vegetative cell is shown in Figure 1-3.

When faced with extreme environmental stresses such as extreme heat, lack of nutrients, or encountering chemicals, many bacteria can rearrange themselves into spores. The spore structure has numerous layers protecting the core housing the cell's DNA. The bacterial spore structure is shown in Figure 1-4. These include an outer proteinaceous coat capable of providing chemical and enzymatic resistance, a cortex composed of a thick layer of peptidoglycan, a germ cell wall, and an inner membrane that acts

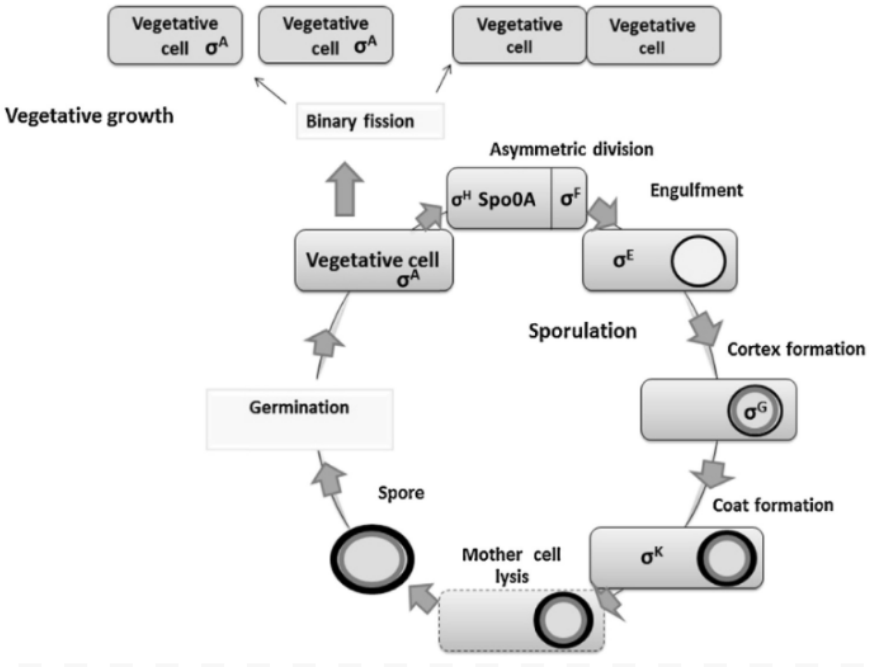

Figure 1-2: The spore forming life cycle of the species *Bacillus*, including the vegetative, spore, and germination phases[5].

Figure 1-3: Scanning electron micrograph of *Bacillus atrophaeus*. The A marks a spore, while the B marks a vegetative cell[5].

as a final barrier to the core. Because of these numerous barriers, spore-form bacteria are extremely difficult to kill, especially with antibacterial agents that would also be

safe on skin.

The outer coat is insoluble and increases the chemical and mechanical resistance of the overall spore. This is due to a large degree of disulfide cross linking between the coat proteins[5]. The inner spore coat is essential for spore formation overall, but has not been shown to provide much additional resistance to environmental stresses[5]. The cortex creates essential components that signal and are used in spore germination[5]. The germ cell wall becomes the new cell wall during spore outgrowth, however, it has not been shown to contribute much to overall environmental stress resistance. The inner membrane has a low permeability to small molecules including water. This protects the core from various chemicals that could potentially damage the spore's DNA. Lastly, the core is at the center of the spore. It houses the spore's DNA, RNA, ribosomes, and many of its enzymes.

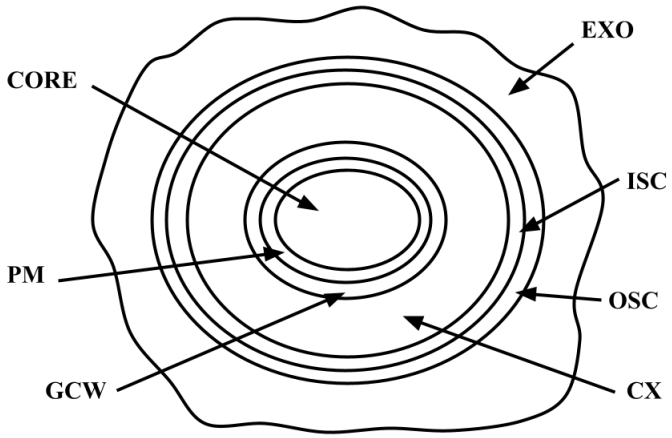

Figure 1-4: A typical bacterial spore depicting the exosporium (EXO), outer spore coat (OSC), inner spore coat (ISC), cortex (CX), germ cell wall (GCW), and the plasma membrane (PM).

The spore forming process takes the form of seven stages. In the first stage, the nuclear material is disposed axially into filaments. In the second stage, a septum (the new cell wall between the two daughter cells) is formed when DNA segregation and

the invagination of the plasmatic membrane in an asymmetric position is completed. Next, the formed septum will begin to curve and the mother cell engulfs the new spore, thus surrounding it with a double membrane. The smaller forespore (endospore precursor) is instead completely contained within the mother cell. Then, the mother cell assists the forespore in full spore development. The forespore assembles the inner and outer proteinaceous layers and the spore cortex is synthesized. In the fifth stage, the spore coat is developed. The coat is synthesized with mother cell deposited proteins. In the next stage, the spore matures and becomes resistant to heat and organic solvents. In the final stage, the mature spore is released when lytic enzymes disrupt the mother cell.

The final phase of the *Bacillus* life cycle is germination. Germination spores show significant resistance to various environmental stresses, such as heat, radiation, pH extremes, and chemicals. They are also capable of remaining dormant for long periods of time, even centuries. Even with an extremely long dormant period, these spores are easily able to continue the cycle should conditions become favorable again. When this happens, the spore can leave this dormant phase and restart growth via germination and out-growth within minutes or hours. The germination process can be defined by three main stages.

The first stage is activation, but this stage is also reversible if needed. First, receptors located in the spore's inner membrane sense favorable environmental conditions. The spore releases H+, K+, Na+, and Ca+2 ions to raise the pH of the spore core. The spore cortex is degraded. In the second stage, the spore core is rehydrated to allow for increased protein mobility and the reactivation of necessary biochemical processes during outgrowth. In the last stage, the vegetative cell is released via spore coat hydrolysis. Germination is followed by outgrowth which further allows the germinated spore to transition into a growing cell. Germination rate varies across different spores and species, and germination is sometimes described as looking like the spore is leaking.

Because spores have a very complicated and robust structure fit with many layers

of protection, many cleansers, including alcohol based sanitizers, are ineffective in killing them. After sanitizer use, the dormant spores can just remain on your hands, as sanitizer has no washing step and can not kill the spores or render them inactive. These dormant spores can remain on surfaces for a long time. They could remain on your hands or surfaces your hands are in contact with. Although spores can remain dormant for long periods of time, conditions could also soon become favorable for the remaining spores to enter the germination part of the life cycle. This would then allow the spread of the corresponding bacterial respiratory or diarrheal illness. For these reasons, if a hand cleanser can not kill spores based on its composition alone, it is imperative that throughout its use the spores are removed.

1.3 Past Work

Much of the prior work done to determine effective water-minimal hand cleansers has focused on one of two things: 1) integrating sanitizers into communities lacking water for sanitation, or 2) formulating new sanitizers with various active ingredients that are comparable to soap and water or ABHS.

Numerous field studies have shown that communities readily adapted to using alcohol based hand sanitizers rather than soap and that hand sanitizers proved comparable in effectiveness to soap[6-8]. One study in Dar es Salaam, Tanzania found that ABHS performed as well as or better than soap and water handwashing at protecting against *E. coli* and fecal *streptococci*[6]. Soap and water handwashing seemed to be more effective against *E. coli* than fecal *streptococci*, while the ABHS reduced the levels of each comparably[6]. The study also found that respondents generally had positive reactions to the use of ABHS despite never seeing a similar product in Tanzania. Many participants also reported that the sanitizer was easier to use, felt better on their hands, and felt that sanitizer was more effective in cleaning their hands than soap and water. There are, however, a few limitations to this study. Firstly, *E. coli* is a non spore-forming, gram negative bacterium, and fecal *streptococci* is a non spore-forming gram-positive Lancefield group D *streptococci* and thus easier to kill.

Although ABHS proved just as if not more effective than soap against these specific bacteria, the ABHS is not proven to provide any protection against various harmful spore formers[2,3]. Additionally, some Muslims are reluctant to use alcohol containing products, including Purell and other ABHS, an important note to consider when choosing a hand wash to distribute[6]. Furthermore, at the time of the study, alcohol-based hand sanitizer was not yet produced at affordable rates in Tanzania or other developing countries. Although ABHS is not difficult to manufacture, it is important to consider the financial viability and potential economic impact of promoting hand sanitizer as a primary hand hygiene option in developing countries.

Another study in Nairobi, Kenya focused on handwashing behavior in schools[7]. Each school underwent a hygiene intervention. These consisted of an initial teacher training session followed by the installation of soap or sanitizer wall dispensers. The teacher training session consisted of a participatory discussion with teachers on germ theory and hand hygiene, a demonstration and practice of correct and effective handwashing/sanitation methods, and the distribution of a student hand hygiene promotion kit that included posters, stickers, a classroom activity book, and a DVD presentation on handwashing with a promotional song. Each school received two dispensers (some schools received liquid hand soap and some received sanitizer). One dispenser was installed next to the bathroom while the other was installed near the eating area. Soap schools also received a water tank. All of the interviewed teachers and a majority of students at sanitizer schools said that they preferred it to soap. Post intervention, schools had a difficult time collecting enough water to continue the rate of hand washing. Unfortunately, this and many other studies did not have a long-term follow-up to understand long term hand washing behavior and long term effectiveness of the hand washing interventions. Even if an effective hand washing method is determined, it will not prove successful if it is not integrated into the community's long-term behavior.

Many studies focus even more on behavioral changes and education to determine how to best integrate different hand washing methods into communities long-term. A study in Dhaka, Bangladesh emphasized using the stages of change theory as a basis

for their intervention program[8]. This theory is used to better understand the factors that influence human behavior and provides steps to promote behavioral change. The stages are 1. Precontemplation, 2. Contemplation, 3. Preparation, 4. Action, and 5. Maintenance. The first stage corresponds to people who are unaware of or have limited awareness of the problem or lack insight into the consequences of their negative/addictive behavior. The second stage corresponds to an awareness and acknowledgment of the problematic behavior with serious consideration to change. The preparation stage is when people prepare to change their habit. The action stage is where the change happens, and the maintenance stage corresponds to a continuation of the new behavior (after six months). In the Bangladesh study, field workers shared knowledge of key handwashing times, pictures of petri dishes showing bacterial growth of contaminated hands, and discussed the relationship between handwashing practice and community/child health. The goal of this was to help move people to the contemplation stage from the pre contemplation stage. Then, bar soap and sanitizer was introduced and placed in convenient locations, and field workers demonstrated how to use the various hand washing products. This was intended to help move the community members into the preparation and action stages. Field workers also encouraged community members to support each other to improve overall hand hygiene. Posters were also placed in key locations. This strategy proved effective in increasing hand washing frequency. Households that received the soap and handwashing promotion increased their frequency of handwashing by 20%. This study again did not investigate the maintenance stage or the hand washing intervention's long-term impact. Even field studies that have examined bacteria capable of forming spores did not examine hand wash viability against the spore form directly.

Ultimately, there are numerous additional factors that need to be taken into account before naming a hand wash effective. Firstly, its potential economic impact needs to be evaluated. Secondly, people need to feel comfortable using the product and with the product's ingredients. Lastly, the product needs to be easily translated into people's daily lives and routines.

Chapter 2

Methods

The goals of the initial experiment were to determine: 1) To which degree soap and other hand washes' compositions alone are capable of rendering spores ineffective. 2) Whether changes to sanitizer use can significantly increase its ability to kill or remove spore forming bacteria. These changes will involve the addition of a particulate during use (such as pumice, salt, or sand), coupling use with a rinse step, and the use of povidone iodine and other non alcohol-based soap recipes.

Each test was run with spore-forming *Bacillus subtilis* bacteria (in spore form) purchased online from Millipore Sigma. The chosen pathogen needs to be spore-forming or non-enveloped in order to compare the performance of ABHS, soap and water, and other cleansers appropriately. Furthermore, this chosen spore-forming bacteria is not pathogenic.

1) The first experiment is intended to test whether soap has inherent pathogen resistance due to its composition that other substances do not. This was performed using a Zone of Inhibition (Kirby-Bauer) test. Nutrient rich agar plates were purchased from Carolina Biological Supply. A sterile swab was used to spread 0.25mL of the *Bacillus subtilis* bacteria across four separate agar plates. Filter paper was cut into squares. One of the squares was coated with Dove bar soap, another was submerged in Purell hand sanitizer, and a third was submerged in Betadine antiseptic solution. Each square was placed at the center of one bacteria-containing agar plate. The

fourth agar plate was used as a control where the filter paper was simply soaked in distilled water. The plates were held at room temperature for 24 hours. If the bacteria was susceptible to one of the paper soaked substances, then a zone of inhibition was seen around where the paper was placed. If the bacteria was resistant to one of the antimicrobial agents, then no zone was evident. A larger zone of inhibition meant that the bacteria was more susceptible to that antimicrobial agent. The sizes of the zones of inhibition allowed for direct comparison between the cleansers' effectiveness in preventing bacterial growth. The zone was measured by measuring the inhibition of each side of the square and taking the average for each side.

When performed as described above, the Kirby-Bauer test does not necessarily indicate that the antimicrobial substance has killed the bacteria, rather that it prevented the bacteria from replicating. This is agreed upon by the Microchem Laboratory. Additional tests were performed to more directly determine each antimicrobial substance's ability to kill the bacteria. The test was performed as described above, except that the *Bacillus subtilis* was swabbed and left at room temperature for 24 hours prior to the addition of the soaked paper in order to first allow the bacteria to grow on the agar.

There were potential issues that needed to be considered when analyzing the data of this experiment. For example, ABHS can evaporate from the filter paper/agar. The Dove soap may also not be absorbed well enough into the agar medium. Because of these concerns, another test was performed in order to confirm and compare the results of the first tests. This last test utilized dilutions to determine the effectiveness of each composition in killing spore forming bacteria. Spore form *Bacillus subtilis* was diluted in the presence of each cleanser. 50% *Bacillus subtilis* spore to dilutant volume ratios were utilized. Because hand washing and the use of hand sanitizer should take less than a minute to be effective, once the bacteria/dilutant dilutions were made, the vials were stirred for 60 seconds before 0.25 mL was then swabbed onto nutrient rich agar plates and grown at room temperature for 24 hours.

2) The second experiment was intended to test whether simple changes to marketed

hand cleaning products can greatly increase their ability to kill or remove spore forming bacteria. The procedure for data collection in this experiment was modeled after a prior experiment done to compare hand cleansers[9]. Cut rawhide rectangles were used to resemble skin and to safely simulate hand washing throughout the experiment. A baseline measurement was first gathered. The rawhide rectangles were washed with 1g of Dove control bar soap and rinsed with water with technique as closely resembling hand washing as recommended by the CDC as possible. Then, 0.5mL *Bacillus subtilis* in spore form purchased online from Millipore Sigma was placed onto the rawhide. The bacteria was spread across the rawhide rectangles and allowed 2 minutes for drying. The rawhide was then placed in a fitted plastic bag and 5mL of distilled water was added. The rawhide rectangles were massaged through the plastic bag for one minute. The liquid from the plastic bag was then drained into a sampling tube, and 0.1mL was swabbed on nutrient rich agar plates and stored at 37°C. After 24 hours, the bacteria colonies were counted. Higher bacterial coverage percentage after 24 hours corresponded to a less successful hand cleaning method.

This bagging process was repeated to test various hand washing methods. However, before bagging, the rawhide was washed with various cleansers. The cleansers tested were Dove control bar soap, Purell hand sanitizer, Betadine antiseptic solution, Purell coupled with pumice, a homemade sunflower oil and commercial KOH based soap, and a wood ash paste. The Purell hand sanitizer alone was tested with and without a follow-up rinse. All other methods were only tested with a follow-up rinse. Hand washing methods were assessed first by visually comparing the number of colony forming units seen in the agar stored at 37°C after 24 hours. Then, a combination of ImageJ software and image processing in Python was used to determine the percent of bacterial coverage on each plate. This experiment provided insight as to whether simple alterations to the usage of marketed sanitizers can significantly increase their ability to kill or remove spore forming bacteria from hands.

Chapter 3

Data and Results

The first experiment performed was the zone of inhibition test. Initially, the *Bacillus subtilis* bacteria was grown in the presence of the cleanser-soaked filter paper square. This test provides indication as to whether the cleanser prevents the growth of the *Bacillus* spores. The cleansers tested were a distilled water control, Purell, Betadine, and Dove bar soap. This experiment was repeated three times for each cleanser. Figure 3-1 below shows the measured zone of inhibitions for each cleanser for each of the three trials. The zone of inhibition was measured from each side of the cleanser-soaked filter paper and the average zone of inhibition value was calculated. Figure 3-2 shows a visual comparison between two of the samples: the first distilled water trial and the first Betadine trial.

Both the distilled water control and the Purell did not prove effective against stopping the growth of the bacteria in their presence. There was no zone of inhibition seen for these cleansers. Betadine and Dove both provided some resistance to the bacterial growth, and a zone of inhibition was seen. The first Betadine test was the most effective overall, as it had the largest inhibition zone of 2.61mm on average. Dove was the most effective cleanser on average, as the average zone of inhibition measured across the three trials was the largest for Dove.

This version of the zone of inhibition experiment was intended to test each cleanser's ability to halt bacterial growth. An additional zone of inhibition experiment was

Cleanser	Side 1 (mm)	Side 2 (mm)	Side 3 (mm)	Side 4 (mm)	Average (mm)
Distilled water (all)	0	0	0	0	0
Purell (all)	0	0	0	0	0
Betadine 1	2.60	1.98	2.77	3.09	2.61
Betadine 2	0.83	1.05	0.84	0.85	0.89
Betadine 3	0.92	0.90	1.06	1.08	0.99
Dove 1	3.01	1.83	2.07	2.87	2.45
Dove 2	2.10	1.12	2.17	0.29	1.42
Dove 3	3.40	1.56	2.40	2.56	2.48

Figure 3-1: The measured zone of inhibitions for each cleanser. The distilled water control and Purell tests were grouped into one row, as the results for each test were the same.

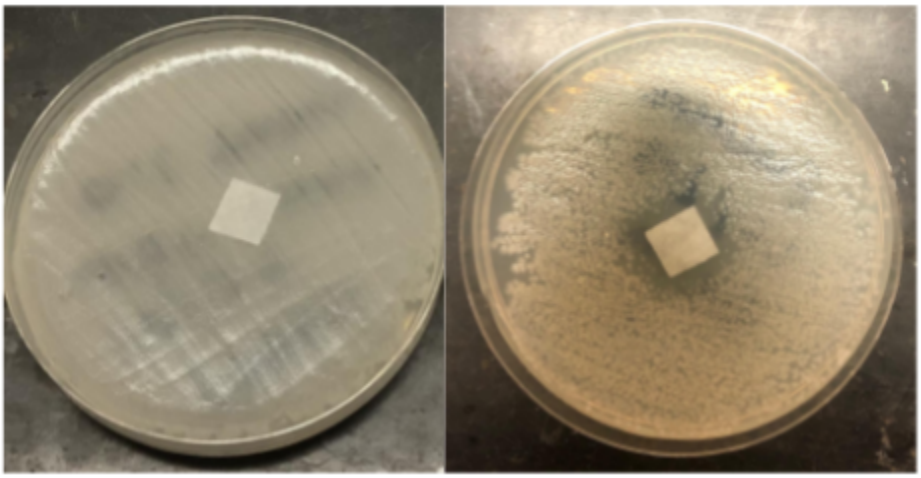

Figure 3-2: Agar dish results for the first zone of inhibition test where the bacteria grew in the presence of the cleanser soaked filter paper. Left: Agar dish for the first distilled water control test; Right: Agar dish for the first Betadine test.

performed to instead test each cleanser's ability to kill the bacteria. To do this, the bacteria was first grown on the agar dishes before the cleanser soaked filter paper was added. In addition to the filter paper, some of each cleanser was also placed directly onto the agar dish. After 24 hours, the agar dishes were examined for zones of inhibition. Figure 3-3 shows a visual comparison between final agar dishes for

Dove, Betadine, and Purell.

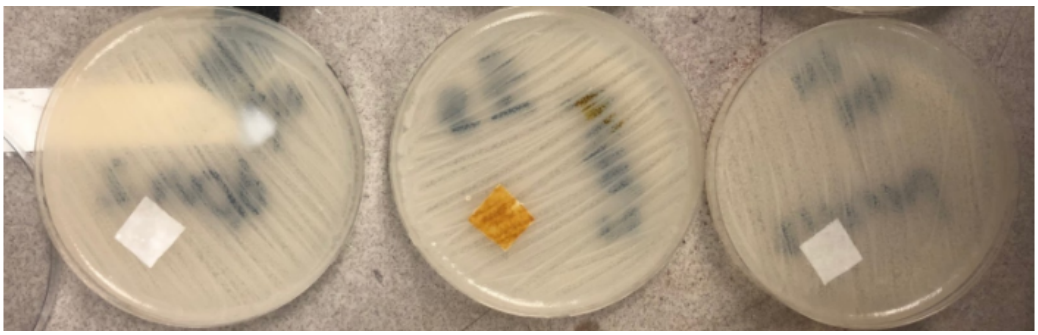

Figure 3-3: Final agar dishes for pregrown *Bacillus subtilis* introduced to various cleansers after growth. From left to right the cleansers tested were Dove, Betadine, and Purell.

No zone of inhibition was seen for any cleanser for both the filter paper and the cleanser placed directly on the agar. This means that no cleanser was shown to be effective in killing the bacteria post growth even though both Dove and Betadine showed some ability to halt the bacterial growth process. An additional experiment was performed to further test this claim. The bacteria was mixed with each cleanser using a 50% cleanser to bacteria volume ratio. The bacteria and cleanser mix was swabbed onto agar dishes and allowed to grow. Cleansers corresponding to agar dishes with less bacterial growth should be more effective in killing the bacteria. Figure 3-4 shows a visual comparison of the post bacterial growth agar dishes for each cleanser.

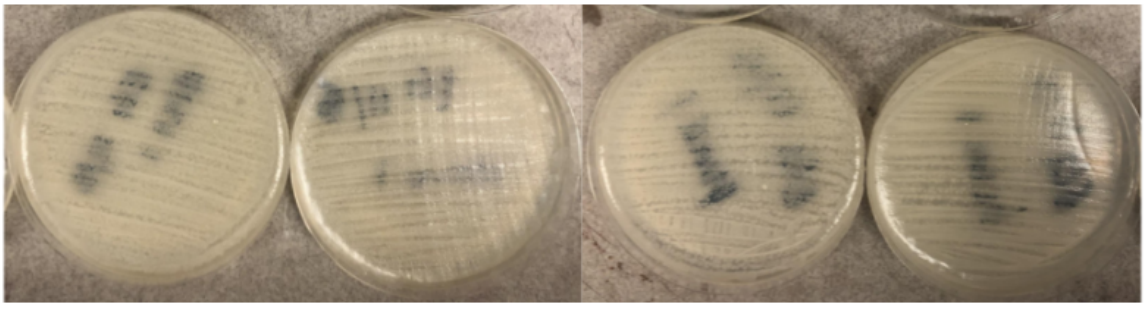

Figure 3-4: Agar dish results for the growth of 50% bacteria to 50% cleanser by volume. From left to right the cleansers used were distilled water control, Dove, Purell, and Betadine.

Across trials for all cleaners and the distilled water control, the agar dishes looked the same. No cleanser alone seemed to significantly reduce the concentration of bacteria by killing it. This further confirms the conclusion that although some of the cleansers can reduce bacterial growth, they are not as effective in actually killing the bacterial spores or rendering them inactive.

The final experiment performed aimed to test each cleanser's ability to remove bacterial spores during a simulated hand wash. The cleansers tested were a distilled water control, Dove, Purell, Betadine, a wood ash paste, two homemade sunflower oil and commercial KOH based soaps, and Purell coupled with pumice. The Purell alone was tested without a follow up rinse. All other cleansers were tested with a follow up rinse. The simulated hand wash was performed, and the remaining liquid was swabbed across agar plates for each cleanser. After allowing 24 hours for the bacteria to grow, the percent bacteria coverage of the agar was calculated for each cleanser. This experiment was repeated three times for each cleanser. The bacteria growth coverage percent results are shown below in Figure 3-5.

Cleanser	*Sample Number*	*Percent Bacteria Coverage*	*Average Cleanser Bacteria Coverage*
Distilled water	1	79.55	75.3
	2	71.05	
Dove	1	42.94	44.14
	2	48.21	
	3	41.28	
Purell	1	62.45	64.99
	2	64.89	
	3	67.63	
Betadine	1	26.95	23.38
	2	17.47	
	3	25.71	
Purell + Pumice	1	17.51	15.61
	2	12.06	
	3	17.27	
Wood Ash Paste	1	37.29	36.32
	2	32.05	
	3	39.61	
Sunflower oil + KOH (1)	1	43.58	52.01
	2	61.16	
	3	51.29	
Sunflower oil + KOH (2)	1	46.23	44.35
	2	46.94	
	3	39.89	

Figure 3-5: The calculated percent bacteria coverages for each cleanser and trial.

As expected, the distilled water control did not prove very effective in removing bacterial spores throughout washing. Likewise, the Purell was also unsuccessful and showed a similar amount of bacterial coverage when compared to the control. The bar soap cleansers (Dove and the two sunflower oil and KOH based cleansers) seemed to be more effective in removing spores throughout washing than the control and Purell. They showed about 30% less bacterial coverage than the control wash. The wood ash paste, Betadine, and Purell with pumice cleansers were the most effective in removing the bacterial spores. The wood ash paste showed about 40% less bacterial coverage than the control, while the Betadine wash showed about 50% less, and the Purell and pumice showed about 60% less. The Purell with pumice was the most effective cleanser overall. Simply adding pumice to the Purell led to a cleanser that was much more effective in spore removal (about half as much resulting bacteria coverage when compared to Purell alone).

Chapter 4

Discussion and Future Work

Based on the results from the above experiments, cleansers that act as a scrub are the most effective at removing spores from the surface of hands. No cleanser tested was particularly effective in killing or preventing the growth of spore forming bacteria based on composition alone. Once a scrubbing and rinsing step was introduced, however, the cleansers became more effective. Purell, often regarded as ineffective in spore removal, became the most effective cleanser once pumice and a brief rinsing step was added.

A combination of Purell and pumice is not the ideal solution for a water minimal hand cleanser as it still requires a brief rinse step to fully remove all pumice residue from the hands. A more ideal solution would be completely water free. Additional work can be done in this area to determine a more optimal overall cleanser.

One possible solution to this issue would be to look into using a cleanser in the form of wet-wipes. Wet wipes would not only allow the user to emphasize the scrubbing step of the hand washing process, but would also require no water throughout use. Firstly, experiments could be performed to test various already-on-the-market wet wipe hand cleansers and compare their effectiveness against Purell coupled with pumice and a brief rinse. To increase the scrubbing ability of the wipes, a particulate could be embedded into the wipe itself. Furthermore, research could be done to determine the optimal average particulate size. The ideal particulate size would be easily embedded

into the wipes, effective in removing spores, and would not harm or damage the surface of hands. If the particulate was too large or jagged, this could lead to cracked or dry skin. A roughened hand surface texture can make spore removal more difficult. Furthermore, large or jagged particulate could lead to a reduction in the hand's natural "resident flora", causing an overall reduction in the body's natural ability to protect against pathogens.

Using a wet wipe approach as a solution to this problem would come with additional complications. Firstly, unlike in soap and water or ABHS washing, wet wipe washing would result in non-biodegradable waste. Should wet wipes first prove to be effective in spore removal, measures would have to be taken to ensure that proper disposal of them would be possible before they could be recommended as a cleanser. Furthermore, if embedding particulate into the wipes significantly increases the wipes' effectiveness in spore removal, the waste the particulate itself can cause would need to be considered to determine if the addition is worth the cost. The easiest way to mass-embed particulate into wipes would likely be to use microplastics, as commonly used in many commercially available beauty products and wipes. Microplastics are extremely harmful to marine environments and are not biodegradable. Other solutions would need to be investigated to avoid further harming available water supplies in already water-lacking areas. Due to these reasons, a wet wipe approach may not prove to be an optimal cleanser for spore removal. Further research would have to be done to confirm this.

There are additional criteria that all cleansers would have to meet before being widely adopted by communities lacking water for sanitation. Firstly, each ingredient would have to be considered to ensure that people feel comfortable using the cleanser. As mentioned earlier, some Muslims are reluctant to use alcohol containing products. Ideally, an effective alcohol-free cleanser or wipe could be found to include all members of the community. Secondly, the cost to produce or import the cleanser would need to be considered. An ideal cleanser would provide an opportunity for local community members to turn cleanser production into an industry without causing any financial

burden to the community. Recommending a cleanser that could not be manufactured locally, but instead had to be imported would be a tremendous disservice to the communities lacking water for sanitation. The health of these communities should not have to be dependent on numerous external entities. Lastly, a recommended cleanser would have to be easily integrated into the lives of the community members. This would mean considering factors such as not having an unpleasant smell, color, or texture, being easy to use, and not requiring a significant amount of time to use effectively. A large ongoing research area is centered around human behavior and adoption of new habits. Developments in this research area would be greatly beneficial when it comes to integrating an unfamiliar hand cleanser into various communities.

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