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BIO 204

Analyzing the Efficacy of Natural Baby Soaps for the Elimination of Bacterial Colonization on Bath Toys

ABSTRACT

Bacteria are one of the most abundant life forms in the environment. These micro-organisms can be located anywhere, including bath toys that children may utilize. Although many may believe that the bath toys are cleaned due to the water and sap in the bath, studies have discovered that these water-retaining bath toys are a base for the formation of bacterial biofilms. These studies demonstrate that the biofilm formation can be influenced by the nutrients in the care products, such as baby soap. Unfortunately, very little is known about the interaction between bacteria on bath toys and baby soaps. Therefore, this research investigated the influence that organic and non-organic baby soaps have on the growth of *Escherichia coli* on rubber ducks. It was hypothesized that organic baby soaps would be more efficient at eliminating bacteria due to the natural antibacterial properties in some of its ingredients. In order to investigate the efficiency, five rubber ducks were coated with *E. coli* and then were washed with their respective soap. Colony formation assays were used to evaluate the efficiency of each soap at preventing the colonization of *E. coli* on the rubber ducks. Through this procedure, it was found that the organic baby soaps were more effective in preventing bacterial colonization. Potential infections in children can be prevented and environments could be sanitized properly by having an efficient soap that will eliminate bacteria effectively.

INTRODUCTION

Water-retaining bath toys have been known to harbor an elevated amount of potentially pathogenic bacteria, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa.* In the study, "Multi-resistant *Pseudomonas aeruginosa* outbreak in a pediatric oncology ward related to bath toys," Jim Buttery and Samantha Alabaster discovered that the bath toys in a pediatric oncology ward were a likely source of the Pseudomonas *aeruginosa* outbreak (Buttery et al., 1998). They discovered colonies of *P. aeruginosa* in the toys and their retained water that genetically matched the bacteria infecting the patients. Unknowingly, bath toys are promoting the growth of bacteria in the environment. However, it is important to consider factors in the environment that may influence the formation of biofilms on and within the water- retaining bath toys. A recent study revealed that biofilm formation on bath toys may be influenced by non- specified organic and non-organic nutrients introduced to the water from care products, such as shampoo, body washes, etc. (Neu et al., 2018).

However, the effects of care products such as shampoo, body washes, etc. on biofilm formation and bacterial growth in bath toys have not been thoroughly explored. In the study, "Tea tree oil body wash versus standard care to prevent colonization", Blackwood and Thompson investigated the influence of 5% tea tree oil and Johnson's Baby Soft Wash in preventing methicillin-resistant *Staphylococcus aureus* colonization. Through this study, Blackwood and Thompson did not identify any significant colonization difference and concluded that the Johnson's Baby Soft Wash and tea tree oil were not effective in reducing colonization (Blackwood et al., 2013). This study was conducted in two intensive care units on critically ill adults who were randomized to Johnson's soap or tee tree oil. Therefore, there is a gap in research because this study was conducted on humans and not bath toys. Due to this gap in research, there is an absence in understanding how these care products affect bacterial growth on bath toys and if there are certain products that can reduce or enhance bacterial growth. Since there is not an abundant amount of research on this topic, people are unaware that there are factors that can aid bacterial growth on the bath toys utilized by their children. These bath toys can prompt children to become ill if the bacteria are pathogenic.

In order to continue with the research, we will investigate how organic and non-organic soaps affect the growth of the bacteria *Escherichia coli* on rubber ducks. The idea was that organic and non-organic soaps are both general designations, and both are available for the average consumer to purchase. By exploring the effectiveness of these organic and non-organic soaps, we can identify which bath products can inhibit bacterial growth and biofilm formation and which products enhance it. In this experiment, it is hypothesized that organic baby soaps will create a more opportunistic environment for bacteria to grow on a rubber duck compared to non-organic soaps. We believe that organic baby soaps will be more efficient in eliminating bacteria because they contain a higher source of antibacterial ingredients, such as rosemary and coconut oil.

By conducting research on the efficiency of baby soaps, we will be able to educate society about the importance bath products have on biofilm formation on rubber ducks and other bath toys. Children are getting ill due to infected toys that were not properly cleaned or cleaned with ineffective cleaning products. With further experimentation, effective cleaning protocols, can by produced to decrease bacterial growth on toys that are continuously used by children.

METHODS

Preparation of LB Agar Plates. The agar plates were prepared for the inoculation of *E. coli* and to observe the amount of *E. coli* on the ducks after being cleansed by the different soaps. The agar solution for plates was prepared according to *Table 1* and autoclaved for 15 minutes at 121^{o}C under 60 psi. While the agar solution was being autoclaved, the plate pouring station was set up. We laid out 15 agar plates, obtained a 10mL automatic pipette, and then sanitized our station to create a clean work area. After the Agar solution was autoclaved, 10 mL was pipetted into each plate to create an equal amount of materials in each plate.

Table 1. Preparation of LB Agar Plates

LB Broth	5 g
BactoAgar	3.75 g
Sterile Water	150 mL

Inoculating *E. coli* on a Plate. In order to coat the rubber ducks in *E. coli,* a LB agar place was obtained and labeled with the plasmid name, date, and initials. Using a sterile loop, an *E. coli* colony was obtained and gently spread over a section of the plate. Then, the loop was dragged through the first streak and spread into another section. This step was repeated two more times. After the last streak, the plate was incubated overnight at 37^{o}C.

Preparation of LB Broth. The LB broth was prepared to grow the *E. coli* in a liquid culture to coat the rubber ducks. The LB Broth was prepared according to *Table 2* and autoclaved for 15 minutes at 121^{o}C under 60 psi. Once the autoclaving was completed, the LB broth was placed into the 4^{o}C refrigerator.

Table 2. Preparation of LB Broth

Lactobacilli Broth Mix	11g

Sterile Water	220 mL

Inoculating *E. coli* in Liquid Culture. To utilize the *E. coli* for experimentation, 10 mL of LB broth were added to a 15mL conical-bottom centrifuge tube. Using a pipette, a single colony was obtained from the plate streaked previously. The pipette was ejected into the tube and inoculated at 37^{o}C overnight.

Coating Rubber Ducks with *E. coli* and Washing. After the bacteria grew in the LB broth, it was transferred to a dish. The five rubber ducks were coated with the bacteria in the dish and located into a separate beaker. The soap mixtures were prepared as seen in *Table 3*. After the soap mixtures were prepared, they were aggregated to the beaker and the rubber ducks were washed for five minutes. Once the rubber ducks were cleansed, they were transferred to a towel to dry.

Table 3. Preparation of Soap Mixtures

Duck #1	Johnson's	6mL	150mL of H_2O
Duck #2	Lafe's	6mL	150mL of H_2O
Duck #3	Dr. Woods	6mL	150mL of H_2O
Duck #4	Aveeno	6mL	150mL of H_2O
Duck #5	Control	6mL	150mL of H_2O

UVA Transmittance Once the ducks were dried, they were placed into separate beakers and then placed under a UV lamp in order to identify potential bacterial growth that would be illuminated by the lamp.

Serial Dilution and Plating. After realizing that the UVA lamp did not provide any conclusive results, a serial dilution was planned according to "Dilution and Plating of Bacteria and Growth Curves." After each duck was dry, the five rubber ducks were placed into separate beakers, in which 10mL of LB broth was added. The duck was swirled around in the beaker and the broth was transferred into a 15mL bottom centrifuge tube to incubate overnight. This was completed for the five rubber ducks. Then a serial dilution was performed, as seen in *Table 4*, for each rubber duck.

Table 4. Serial Dilution

	LB Broth	
Dilution 1	900 µL	100µL of Duck # *E. coli*
Dilution 2	900 µL	100µL of Dilution 1
Dilution 3	900 µL	100µL of Dilution 2

After the serial dilution, three LB agar plates were obtained for each duck. In each plate, 100 microliters of each dilution were added and spread onto plates. After the plates were inoculated with the dilution, they were incubated at 37^{o}C for 24 hours.

RESULTS

UVA Transmittance

The rubber ducks were placed under a UV lamp after being cleansed and allowed to air dry in order to observe if the rubber ducks cleansed with baby soap still harbored bacteria. This was done with the intention of being able to quantify the number of bacteria growing on the surface of the ducks. However, this method gave us little to no data. As seen in *Figure 1*, it was not easy to identify bacterial growth on the ducks under the UV light. Therefore, a different approach was utilized to yield results over the experiment.

Figure 1. Cleansed Ducks 1-4 under UVA Lamp.

Serial Dilution and Plating of Bacteria

The serial dilution and plating were utilized to measure the amount of *Escherichia coli* on the rubber ducks after they were cleansed with the assigned baby soap and observe if organic baby soaps harbored more bacteria that non-organic baby soaps. In the serial dilution, duck number five was treated as an indicator of uncontrolled bacterial growth since it was not cleansed. Each serial dilution was inoculated onto agar plates to observe the number of forming colonies to be able to compare each soap.

Run 1. The initial serial dilutions and plating completed demonstrated that the four soaps were capable of reducing the concentration of bacteria on the rubber ducks. As seen in *Figure 2*, rubber duck number five has the highest concentration of *E. coli* on the agar plate since it was not cleansed with any soap. The four cleansed rubber ducks have significantly less bacterial growth on the agar plates of each solution than the uncleansed rubber duck. *Figure 2* and *Table 5* portray that rubber ducks washed with the organic baby soaps have few to no forming colonies. Rubber duck number two, disinfected with Lafe's organic baby soap, portrayed no growth on the three diluted agar plates. Rubber duck number three, washed with Dr. Woods organic baby soap, had two forming colonies in the first dilution and no colonies in the second and third dilution. On the other hand, the rubber ducks cleansed with non-organic baby soaps had multiple forming colonies. As seen in *Table 5* and *Figure 2*, rubber duck number one, washed with Johnson's baby soap, grew 30-40 more *E. coli* colonies than rubber ducks' number two and three. Rubber duck number four, cleansed with Aveeno, had the highest amount of forming colonies out of the four disinfected rubber ducks. Through the plating of the serial dilutions, it can be observed that the non-organic baby soaps preserved higher forming colonies than the organic baby soaps. In this run, it can be concluded that organic baby soaps had a higher efficacy in eliminating bacterial colonization.

Figure 2. Serial Dilution #1 of *E. Coli* Growth on Rubber Ducks

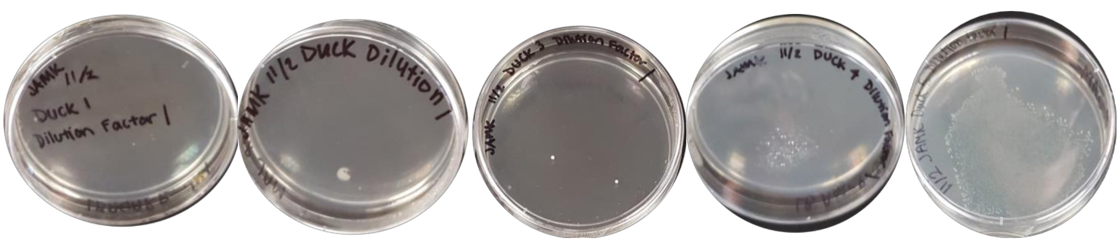
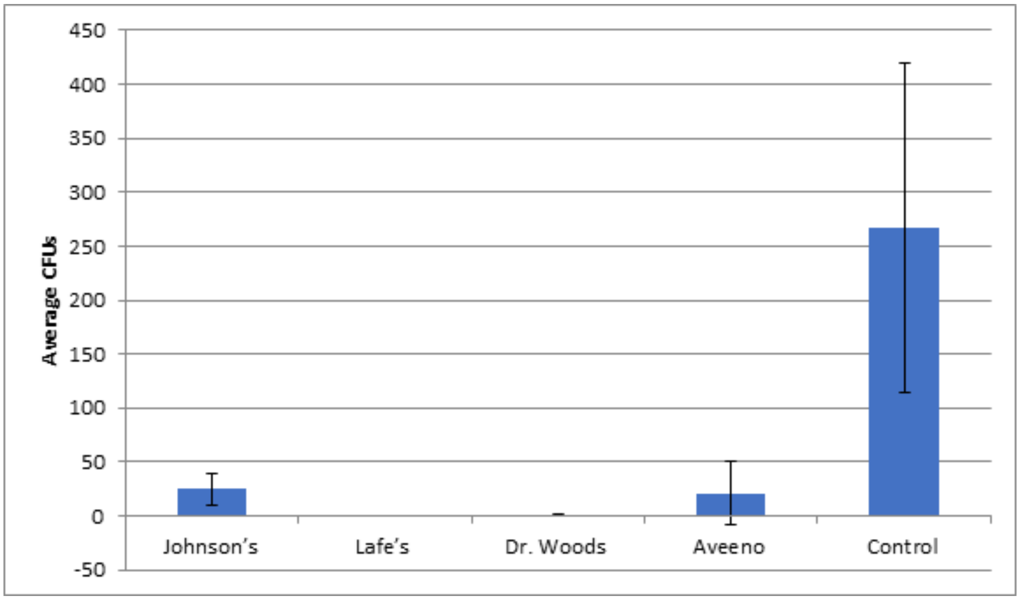


Table 5. Serial Dilution Results

Duck #	Soap Utilized	Colony Forming Units Dilution 10^{-1}	Dilution 10^{-2}	Dilution 10^{-3}
1	Johnson's	40	25	10
2	Lafe's	0	0	0
3	Dr. Woods	2	0	0

4	Aveeno	55	7	1
5	Control	500	300	100

Figure 3. Average Colony Forming Units



Run 2. The second serial dilution and plating reinforced that the four soaps reduced the number of bacteria on the rubber ducks. As seen in *Figure 4*, rubber duck number five has a significantly higher average CFUs. *Figure 4* and *Table 6* portrayed a similar trend as the previous run, but there are important differences. Rubber duck number two, disinfected with Lafe's organic baby soap, portrayed a higher number of colonies than the first run. In the first serial dilution, duck number two had no colonies in any of the dilution. There was a significant increase in the colonies. On the other hand, rubber duck number three, washed with Dr. Woods organic baby soap, had no forming colonies in any of the dilution. In the last run, this duck had two colonies in the first serial dilution and none in dilution two and three. Rubber duck one, washed with Johnson's, also had a significant change. As observed in *Figure 3,* the plate has one merging colony. Unlike the last run, the colonies are merging into one making it difficult to calculate the exact CFUs. However, in the second and third dilution, no colonies are seen. In this run, the CFUs decreased and had less than the organic baby soap, Lafe's. Rubber duck number four, cleansed with Aveeno, demonstrated that same trend. It had less CFUs than the control, but the plates had more bacteria than the ducks washed with the organic soaps and Johnson's. With its consistency, it can be seen that Aveeno may

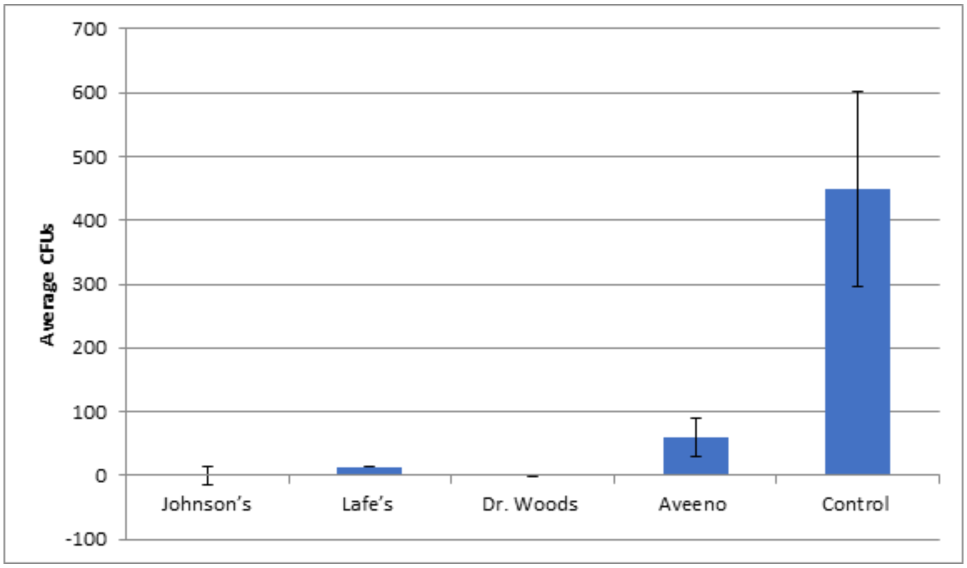
eliminate some of the bacteria; however, it is not as efficient as other baby soaps. In this run, it is unclear which soap is more efficient since Dr. Woods and Johnson's yielded similar results.

Figure 3. Serial Dilutions of *E. Coli* Growth on Rubber Ducks week 2

Table 6. Colony Forming Units

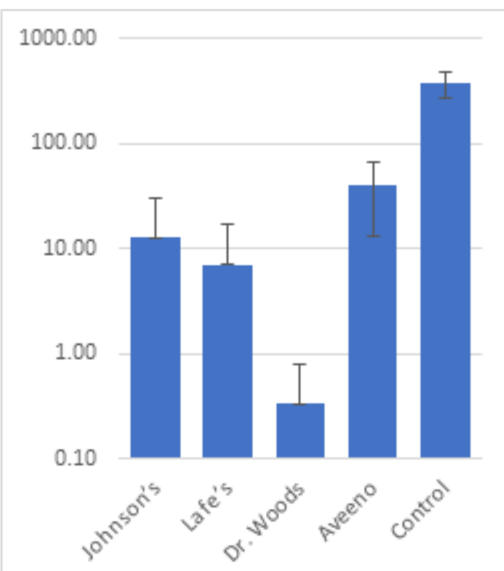
		Colony Forming Units		
Duck #	Soap Utilized	Dilution 10^{-1}	Dilution 10^{-2}	Dilution 10^{-3}
1	Johnson's	1	0	0
2	Lafe's	34	7	1
3	Dr. Woods	0	0	0
4	Aveeno	116	56	7
5	Control	800	300	250

Figure 4. Average Colony Forming Units



As seen in *Figure* 5, run 1 and 2 portrayed that the organic soaps have fewer *E. coli* colony forming units than the non-organic bay soaps. Collectively, there is a significant difference between the efficiency of the organic and non-organic baby soap. Therefore, it can be concluded that organic baby soaps were more efficient in eliminating bacterial colonization.

Figure 5. Overall Efficiency of Baby Soaps



DISCUSSION

Recent studies have discovered that water-retaining bath toys are a base for the formation of bacterial biofilms due to available nutrients from care products. In order to investigate the effect of baby soaps on biofilm formation, we tested how organic and non-organic baby soaps affected the growth of the bacteria *Escherichia coli* on rubber ducks. It was hypothesized that the organic baby soaps would be more efficient in eliminating bacterial colonization on the water-retaining toys.

Initially, the original method designed did not yield concise results. When the rubber ducks were observed under the UVA lamp, it demonstrated a vague number of bacteria; however, it was difficult to record concise data. Therefore, a colony formation assay was completed to generate quantitative data. The results of the colony formation assay affirmed the hypothesis that organic baby soaps would eliminate bacterial colonization more effectively than non-organic baby soaps.

The results of the first run portrayed that the ducks washed with the non-organic soaps, Jonson's and Aveeno, left a moderate amount of bacterial colony forming units, and the Lafe's and Dr. Woods organic baby soaps demonstrated little to no colony forming units. The control duck, which was not washed, produced a large amount of colony forming units. The results of our second run during week 2 were largely consistent. The ducks with the least amount of bacterial growth were ducks 1 (Johnson's, non-organic) and duck 3 (Dr. Woods, organic). Duck 2 (Lafe's, organic) produced several colonies where before there had been none. Duck 4 (Aveeno, non-organic) displayed similar results as the week before. However, the rubber ducks washed with organic baby soaps, collectively, eliminated more bacteria than the non-organic baby soaps

Some of the ingredients in the organic baby soaps have antibacterial properties. This why it was hypothesized that the ducks washed with the organic baby soaps would contain less bacterial colonies. For example, the Dr. Woods baby soap contained sea salt and rosemary extract. A study discovered that rosemary was effective in preventing growth of bacteria, such as *E. coli*, the model organism utilized. Coconut oil, which was an ingredient in both organic soaps and, had also been found to have antimicrobial properties due to the lauric acid (Nakatsuji et al). In *Figure* 5, it can be seen that the organic baby soaps had fewer colony forming units than the control and non-organic baby soaps. Therefore, it can be concluded that the organic baby soaps were more efficient overall because they contained the nutrients with antibacterial properties.

This research allowed us to understand that using any type of baby soap is better than not using any at all although organic baby soaps may be more effective in eliminating bacteria. The data demonstrated that not using any soap resulted in at least 500 bacterial colony forming units. Therefore, it is important to provide families with an effective method to sanitize their bath toys with an efficient soap so bacterial growth is eliminated.

In the future, it would be beneficial to determine specific bacterial species that can be effectively cleaned with the baby soaps utilized in this experiment. It would be interesting if we could identify if there is a specific ingredient in organic baby soaps that are eliminating the bacteria. By identifying this ingredient, it could be aggregated to cleaning products to enhance their effectiveness and eliminate bacterial colonization.

RESOURCES

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