DOES WASHING YOUR FRUIT MAKE A DIFFERENCE?
EVALUATING DISINFECTION METHODS TO REMOVE BACTERIA FROM CANTALOUPE MELON

SEAN BLOW | SRES 2100

Introduction

Bacteria is found throughout the world, from hydrothermal vents in the ocean, the air we breathe, and even in our body (Parker, et al., 2016). Most bacteria are considered harmless and can even benefit us, such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). However, there are approximately 1% that are considered pathogenic (Parker, et al., 2016). Bacteria are split into two groups: Gram-Positive (GPB) and Gram-Negative (GNB). Both GPB and GNB can be spread to humans through contaminated soil, water for irrigation, and handling procedures and some species can cause gastrointestinal illnesses if ingested with a high enough dosage (Gerken, et al., 2022). For example, the most recent outbreak involving cantaloupe melon (October 2023-January 2024) resulting in 9 deaths across Canada. As fruit and vegetables are an important part of a healthy diet it is important to consider removing as many potentially harmful bacteria as possible to avoid any type of illness; particularly in the elderly and immunocompromised. We hypothesized that any method of disinfection on cantaloupe melon would show a difference than the untreated one.

Purpose

The purpose of this study was to gauge the effectiveness of various disinfection methods to reduce the number of potentially harmful bacteria found on cantaloupe melon skin.

Method and Methodology

Collection of Fruits and Vegetables

There were preliminary studies done that tested for the consistent growth of bacteria from multiple types of fruits and vegetables. Cantaloupe melon was chosen due to the moderate growth of bacteria and supported by the recent outbreak in October 2023.

Plating & Growth of Bacteria

The cantaloupe melon was aseptically cut into 4x4 cubes, treated with the multiple disinfectants and plated in triplicates onto tryptic soy agar (Oxoid). Negative control plates of water only, vegetable spray only, tea towel only, and no treatment were plated and *E. coli* were plated as a positive control. They were incubated for 24 hours at 37°C and once removed they were refrigerated until being gram-stained.

Methods of Disinfection

The three disinfection methods were decided based on the availability to the common consumer and low price point.

- Cold water was used from the lab tap and was run for 5 seconds before rinsing the cantaloupe melon piece with 5 seconds between the three samples.
- Vegetable spray, bought online, was used due to the inclusion of a surfactant (Lauryl & Decyl Glucoside) in the ingredients and is purported to remove contaminates such as soil, pesticides, and bacteria.
- A tea towel was used to physically wipe the skin of the cantaloupe as the general public has access to one.

Gram-Staining

Samples from the most common type of bacterial colonies were Gram stained with control bacteria (*E. coli* and *S. aureus*) and viewed under 100x objective (Zeiss microscope)

Results

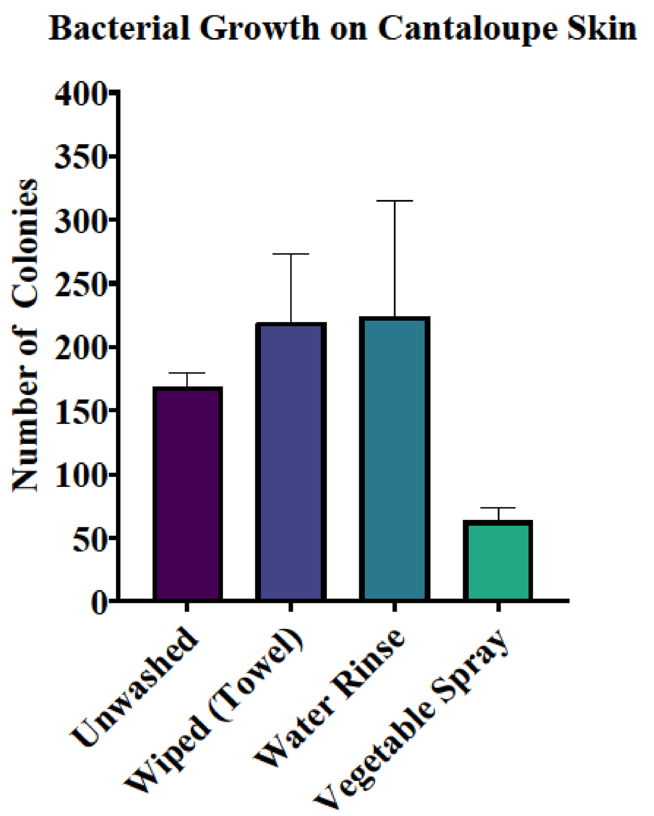

Fig 1A. **Bacterial Growth After Disinfection Treatment.** Data are Mean and SEM of triplicates. Vegetable spray was reduced by 37.5%, whereas the wipe and water rinse increased the bacterial load by 30% and 33%.

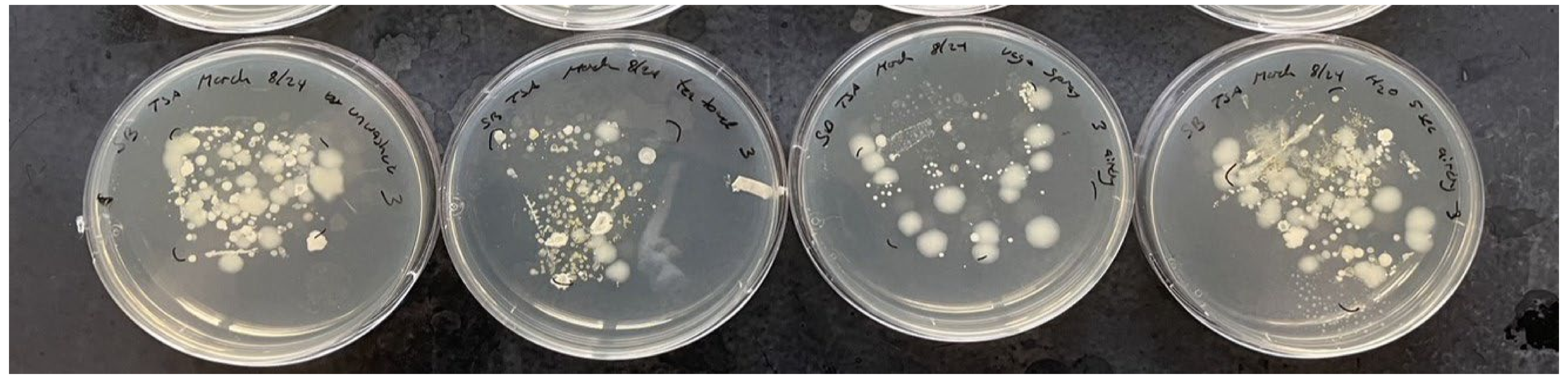

Fig 1**B. Samples of cantaloupe melon printed on TSA and treated with a variety of disinfection methods**. One sample of each triplicate is shown here.
From left to right: Unwashed, Wiped (Towel), Vegetables Spray and Water Rinse

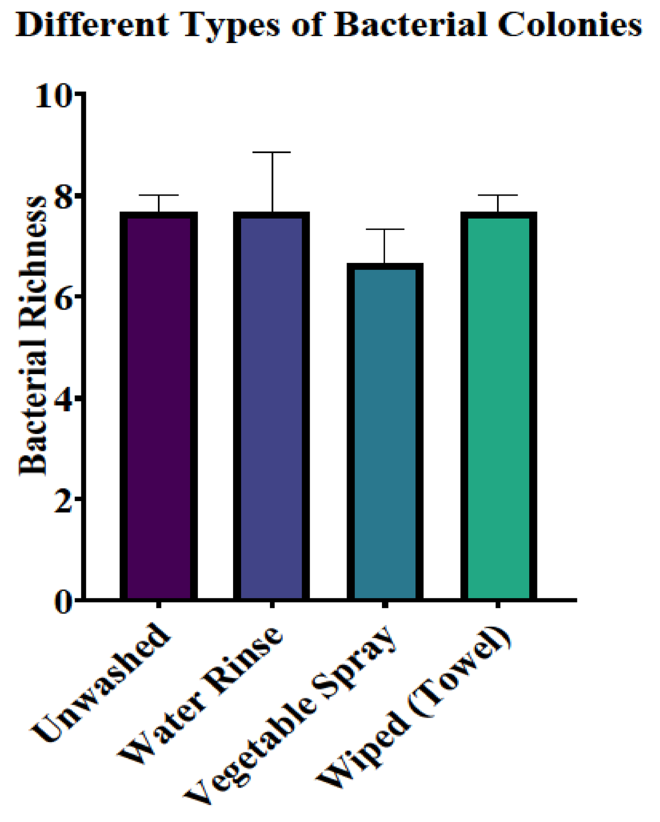

Fig 2. **Number of different types of bacterial colonies (richness) after disinfection treatment.** Disinfection did not affect the number of types of colonies. Data are Mean and SEM triplicates.

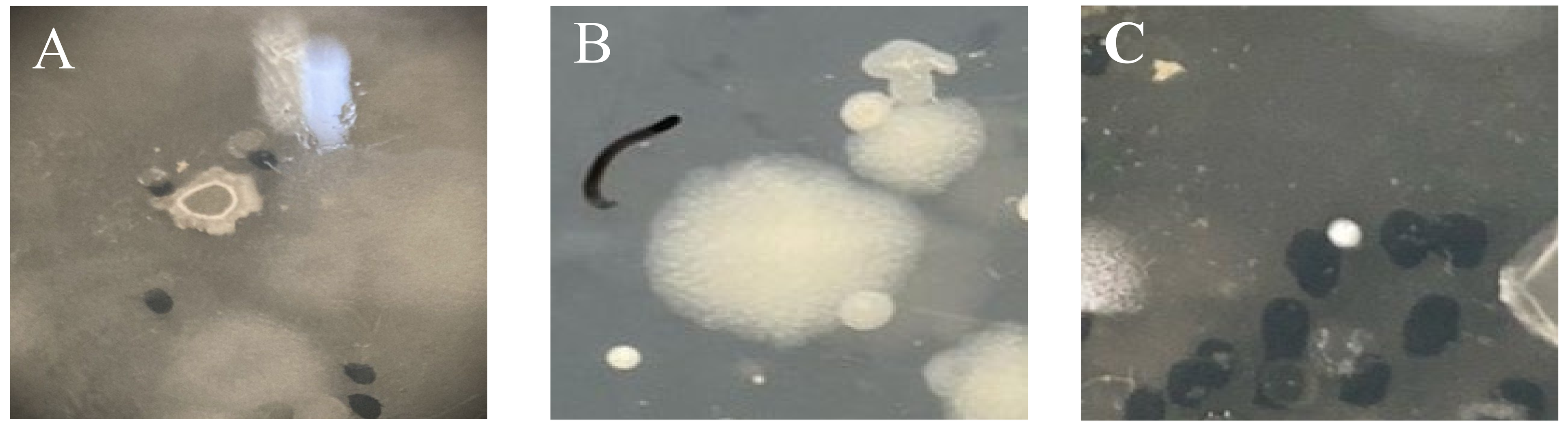

Fig 3. **The three most common colonies on unwashed cantaloupe skin.**
A) Crateriform colony **B)** Lobate colony **C)** Circular colony

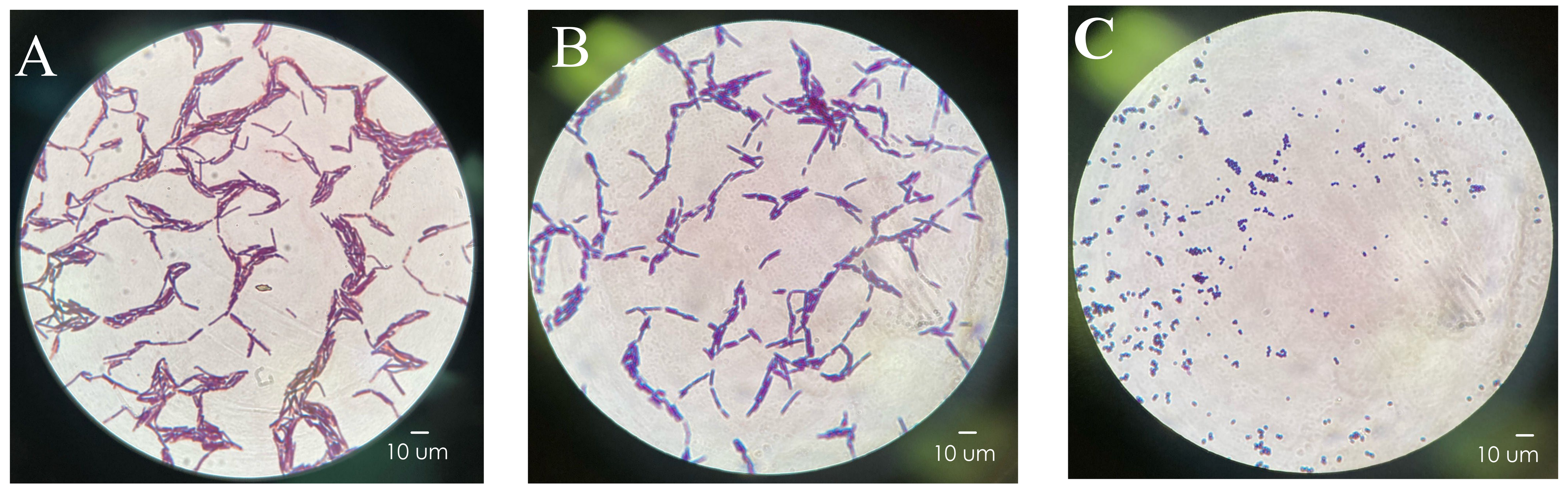

Fig 4. **Gram stain of common colonies A)** Crateriform bacteria are Gram-positive bacilli (100x objective) **B)** Lobate bacteria are Gram-positive bacilli (100x objective) **C)** Circular bacteria are Gram-positive cocci (100 x objective)

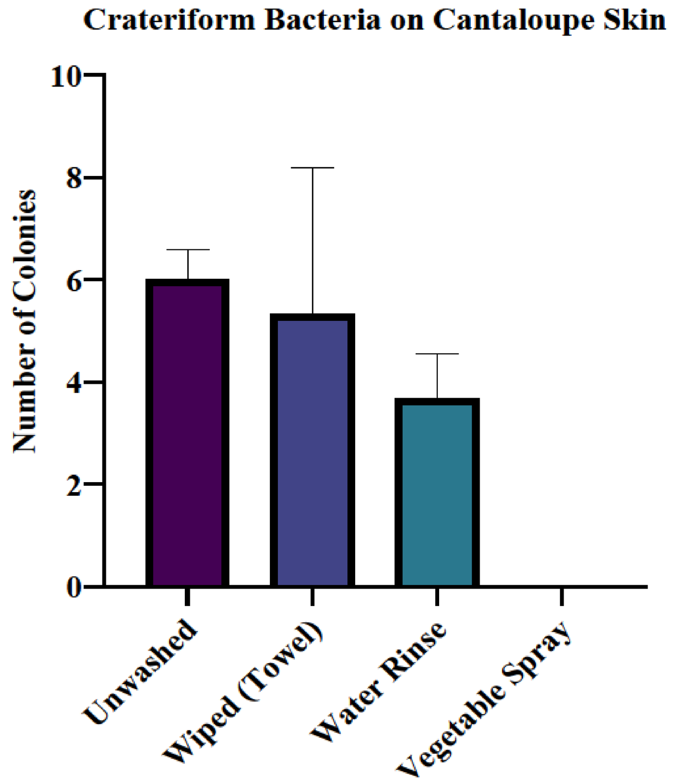

Fig 5. **Growth of Crateriform bacteria after disinfection post treatment.** The vegetable spray reduced the count of crateriform to 0. Data are Mean and SEM of triplicates.

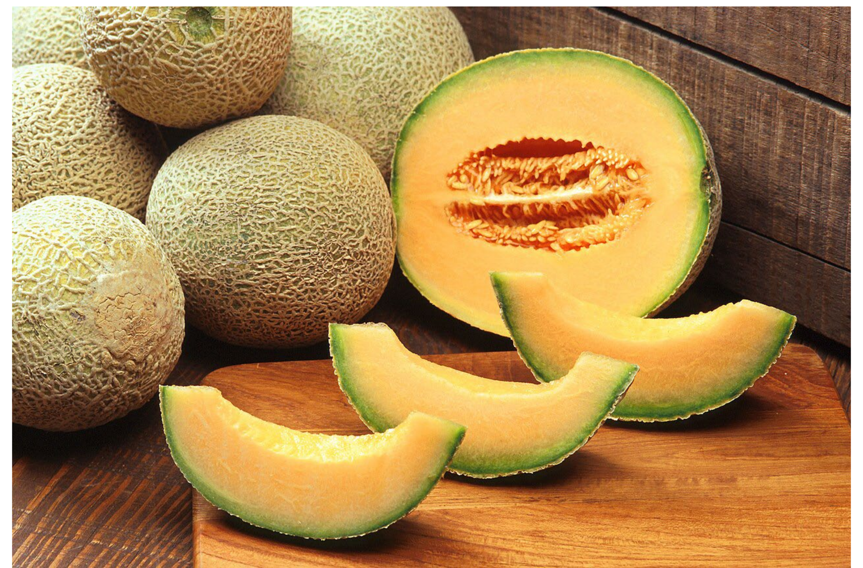

Fig 6. **North American Cantaloupe.** Type of cantaloupe melon used in this experiment (Scott Bauer 2023)

Analysis & Statistics

GraphPad Prism was used to plot data for statistical analysis. The data were collected in triplicate and analysed using one-way ANOVA and post hoc Tukey's test.

Discussion

- Bacteria grew from the melon skin and the vegetable spray was the best at removing it (though not statistically significant with this small sample size.) The water rinse and wipe appeared to increase bacterial colonies. The vegetable spray was most effective at removing crateriform bacteria colonies were characterised as Gram-positive bacilli.
- Possible causes of reduction in bacterial growth using the vegetable spray may be due to the detergent which formed a thin slimy film on the melon that may have assisted in the prevention of transfer to the plate. The surfactants target the cell membrane of the bacteria and interrupts the ETC and possibly cause cell lysis (Falk, 2019)
- All bacteria tested were Gram-positive. It is possible that many Gram-negative bacteria species, such as *Salmonella,* were removed at the processing plant as they submerge the melons in a chlorinated bath.. Examples of possible gram-positive bacteria that can grow in soil and cause gastrointestinal illnesses are *Clostridium perfringens*, *Clostridium difficile*, and *Bacillus cereus* (Lim, et al. 2020; Ohtani & Shimuzu. 2016; Stenfors et al., 2008)
- A possible reason for the common type of bacteria to be gram-positive may be because cantaloupe melons only grow on top of the soil as opposed to in the soil, which could have the possibility of transferring more gram-negative bacteria, such as *E. coli* and *Salmonella*,
- The 33% increase in growth of bacterial colonies from the wipe with the tea towel was expected, as it was not sterilized but a household item.
- The water rinse increase was surprising as water here is treated thoroughly. Thoughts on how an increase of 30% may be explained by poor aseptic techniques, time spent air drying, or cross contamination.
- The crateriform bacteria was the most susceptible to the veggie spray.

Future Direction

For future experiments it should be noted that sample sizes should be larger to incorporate a better understanding of the true reduction in numbers, as triplicates were not adequate in showing a significant difference. Due to time constraints, we were unable to isolate and test all bacterial colonies that were present through gram-staining and DNA extraction to identify which specific bacteria we were dealing with. It would be interesting to perform further tests including DNA analysis to determine which species were present on the plates.

References

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