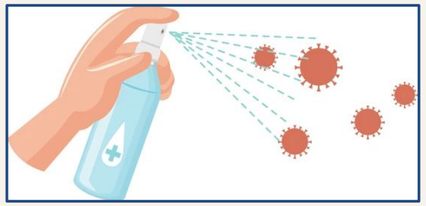
SPRAYING THE FACIAL BACTERIA AWAY

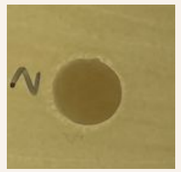
Testing which antimicrobial facial spray is more effective in killing bacteria commonly found on the face.

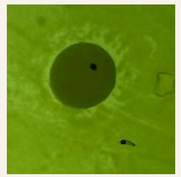
Research Question

How effective are antimicrobial sprays with hypochlorous acid at killing the most commonly found bacteria on the face, staphylococcus epidermidis?

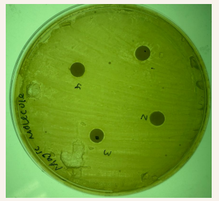


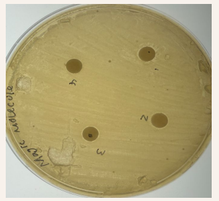
Results

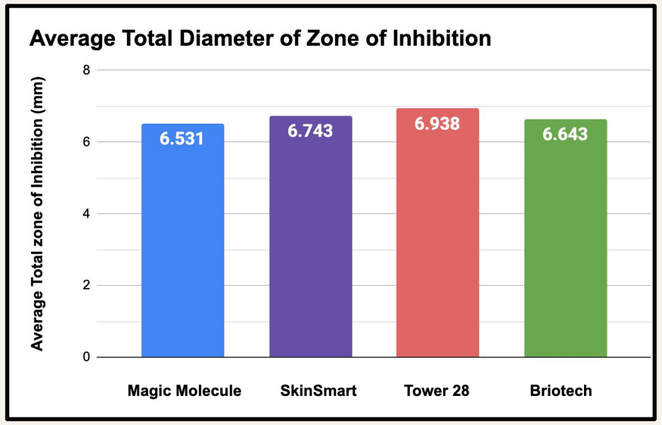




Zone of inhibition







Methodology

- **Prepare and pour Mueller-Hinton agar plates**
  - **Grow Staphylococcus epidermidis on a plate for 24 hours**
- **Soak 6 mm paper discs with each antimicrobial spray:**
- **Preparing Bacteria Lawn**
  - Pick 3–5 colonies from Staphylococcus plate and mix into tryptic soy broth
- **Place dried discs onto the agar**
  - **Invert plates and incubate for at least 24 hours**
- **Measure the zones of inhibition (mm)**

Conclusion

1. My research supported my hypothesis that hypochlorous acid is effective in reducing staphylococcus epidermidis cultures.
2. The most effective brand of antimicrobial spray was Tower 28, average zones of inhibition 6.938 mm.

Introduction

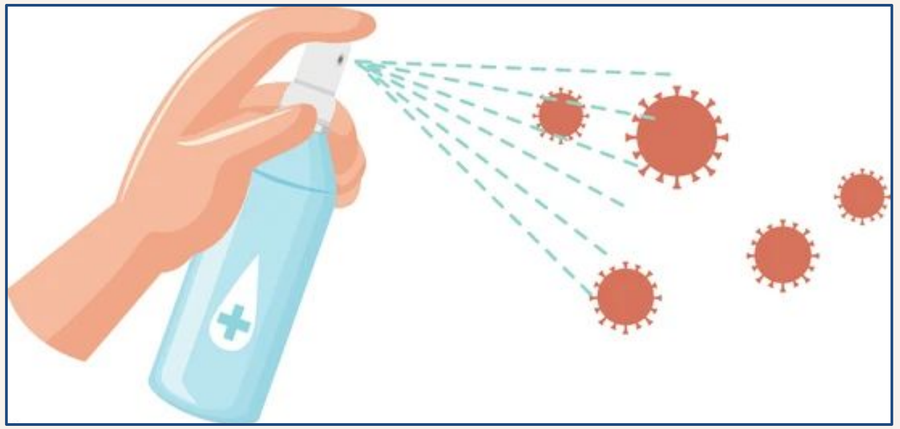
- **Staphylococcus epidermidis**
  - most **commonly** found bacteria on the **skin**: generally harmless, but can contribute to larger issues if growth is not controlled (Claudel et al., 2019)
  - prefers **high sweat** areas and concentrations (Swaney et al., 2023)
  - excessive sweating can disrupt **balance** of the skin's **microbiome**, promoting this bacteria (Niedźwiedzka et al., 2024).

- **Hypochlorous acid**
  - **antimicrobial** effectiveness
  - 0.01% concentration was found to have **reduced** ***Staphylococcus epidermidis*** by over 99% within 20 minutes, without significantly disrupting the overall bacteria diversity (Stroman et al., 2017)

- **Bacteria Lawn and Diffusion Discs**
  - Bacterial lawn with diffusion discs saturated in substance can create **zones of inhibition** representing the **effectiveness** of the substance. (Schotanus & Venables, 2022)

Research Question

Are antimicrobial sprays with the active ingredient being hypochlorous acid effective in killing the most commonly found bacteria on the face, Staphylococcus epidermidis, and which brand is most effective?



Antimicrobial Sprays- Images obtained from official brands websites

Variables and Hypothesis

Independent variables-

- Type of antimicrobial spray used

Dependent Variables-

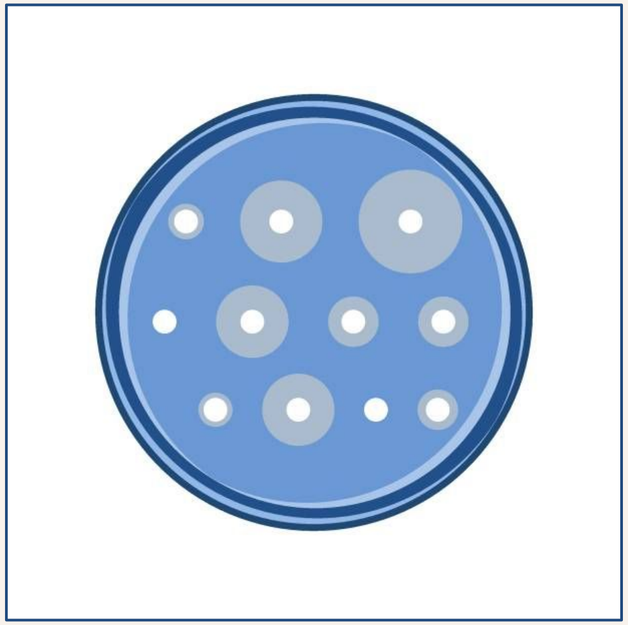
diameter of zone of inhibition (mm)

Hypothesis: Antimicrobial sprays containing hypochlorous acid will inhibit or reduce the growth of Staphylococcus epidermidis, and the spray with the strongest concentration of hypochlorous acid will produce the largest average zones of inhibition.

Control-

Incubating a bacteria lawn with blank discs (no antimicrobial spray) to confirm zones of inhibition are caused by the sprays.

- Amounts of solutions pipetted onto each disc
- Same bacteria (Staphylococcus epidermidis)
- Same concentration (0.5 McFarland standard)
- Evenly spread bacterial lawn on each plate
- Same type and thickness of Mueller-Hinton agar
- Same disc size (6 mm)
- Same incubation time and temperature



Methods

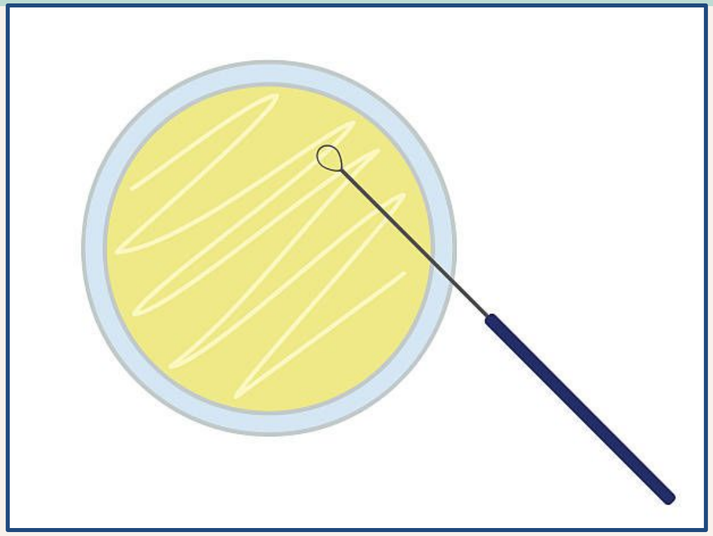
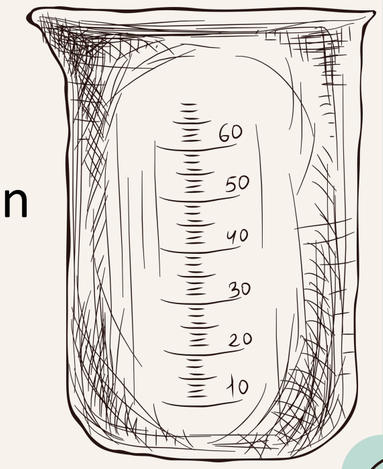


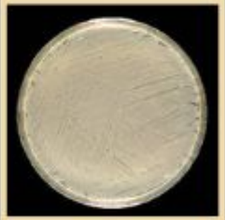
Plate with inoculation loop streaking bacteria

- **Prepare and pour Mueller-Hinton agar plates**
  - Water, agar powder, and Mueller-Hinton broth powder
- **Grow Staphylococcus epidermidis on a plate for 24 hours**
- **Soak 6 mm paper discs with each antimicrobial spray:**
  - Magic Molecule antimicrobial skin spray, SkinSmart antimicrobial facial cleanser, Tower 28 SOS Daily Rescue Spray, Briotech Topical Skin Spray
  - Let discs air dry
- **Preparing Bacteria Lawn**
  - Pick 3–5 colonies from Staphylococcus plate and mix into tryptic soy broth
  - Vortex and adjust to a 0.5 McFarland standard using a spectrophotometer
  - Spread the bacterial suspension evenly over Mueller-Hinton agar to form a lawn
- **Place dried discs onto the agar.**
- **Invert plates and incubate for at least 24 hours**
- **Measure the zones of inhibition (mm)**



Methodology Chart

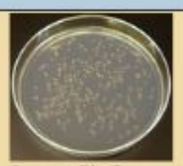
Step 1) Muller-Hinton agar plates



Step 4) Streak and grow the bacteria for a bacteria lawn

Step 5) Pipette 30 ul of hypoclorous acid spray from the different brands onto the difution disc

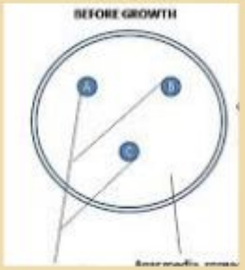
Average the zones of inhibition for each brad of spray



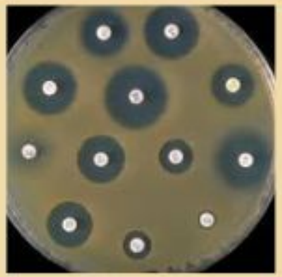
Step 2) Grow staphylococcal epidermis on plate

Step 3) isolate 5 colonies and mixed into a 5 mL vial of tryptic soy broth

- using spectrophotometer ensure liquid matches McFarland standard at 100



Step 6) Place diffusion discs on bacteria lawn



Step 7) Incubate petri dish with diffusion disc

Step 8) Measure diameter of zone of inhibition (mm)

Step 9) Test multiple times

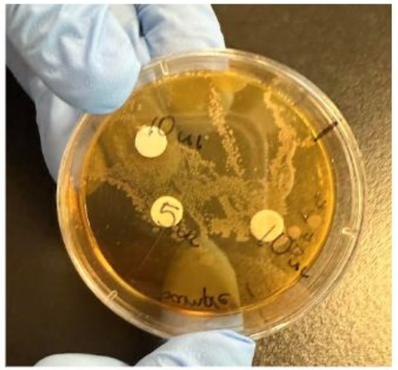
Day	Frequency	Response
0	14	0.09983
1	60	0.40242
2	99	0.66562
3	129	0.86366
4	146	0.97716

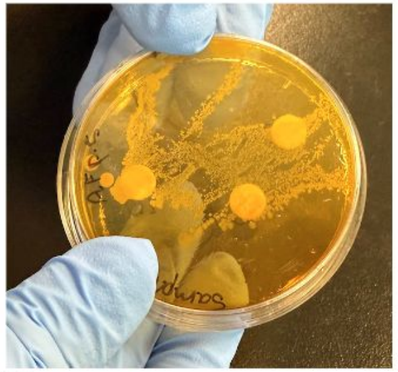
Step 10) Record data

Results: Trial 1 and 2

A)

B)


C)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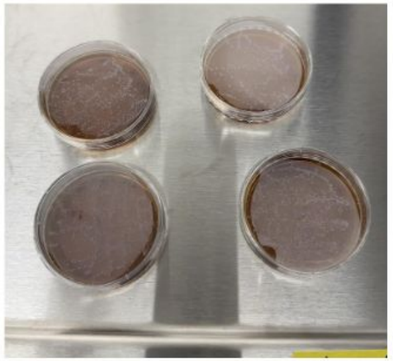
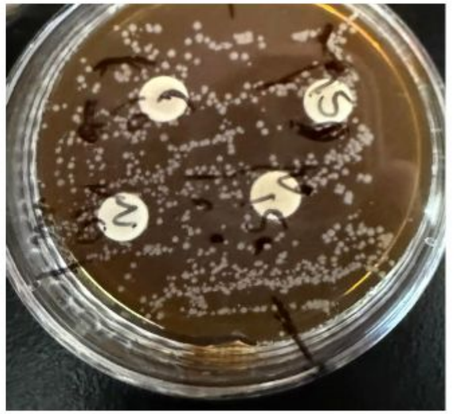

E)

F)

All images contain tryptic soy agar plates with staphylococcus

G)


H)

Trial 1 and 2 before using bacterial lawn

- **A**) and **B**) was first attempt of growing staphylococcus epidermidis bacterial cultures
- **C**) and **D**) plates after incubation with saturated diffusion discs. The results were inconclusive.
- **E**) and **F**) Trial 2 of staphylococcus plates before incubation with discs
- **G**) and **H**) after incubation with discs, bacteria was not concentrated enough to visualize results or zones of inhibition

Results Using Bacterial Lawn

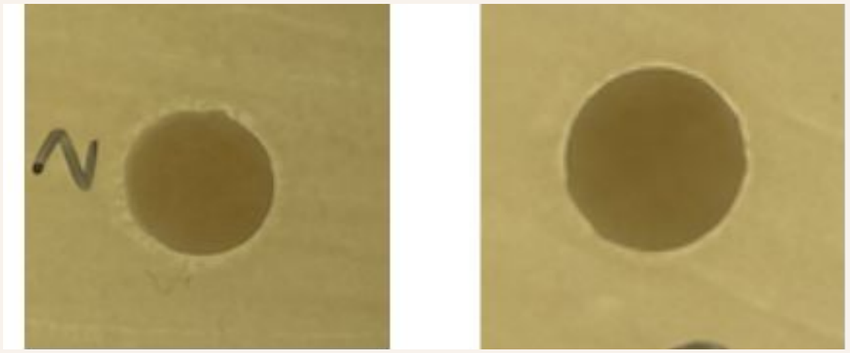
Petri dish with bacteria lawn

Blank discs from Carolina Biological

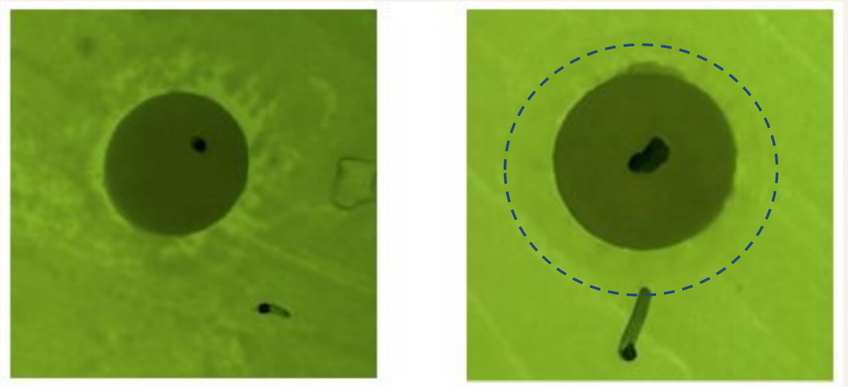
- This table depicts the diameter of the zone of inhibition of each 6mm diffusion disc, measured from one edge to the other edge.
- Represents which brand of spray had the largest average inhibition zones and were most effective in reducing bacteria.

Brand of Antimicrobial Spray	Trial 1 (day 1) Zone of Inhibition Measurements (mm)	Trail 1 (day 2) Zone of Inhibition Measurements (mm)	Trail 2 (day 1) Zone of Inhibition Measurements (mm)	Trail 2 (day 2) Zone of Inhibition Measurements (mm)	Total average Zone of Inhibition (mm)
Magic Molecule	6.1	6.5	7	7.2	
antimicrobial	6	6.5	6.5	7	
skin spray	6.5	7	6.5	6.5	
	6.5	6.5	6.1	6.1	6.531
SkinSmart	7	9	6	6	
antimicrobial	6	7	6	6.5	
Facial cleanser	6.8	9	6.5	6	
	6.1	6.5	No disc 4	n/a	6.743
Tower 28	6	7	6	8	
sos daily	7	7	7	6	
rescue skin	6	8	7	7	
spray	8	9	6	6	6.938
Briotech Topical	6	6.2	6.1	6.1	
skin spray	6.5	7.5	6.1	6.8	
	6.5	7	6.5	6.2	
	8	7.5	No disc 4	n/a	6.643

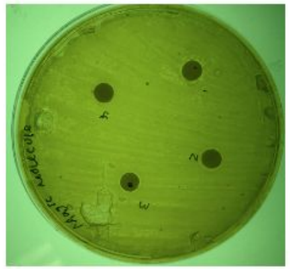
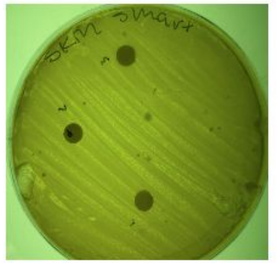
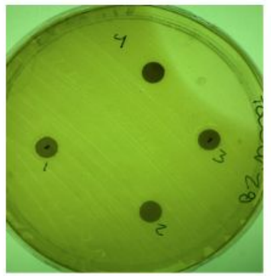
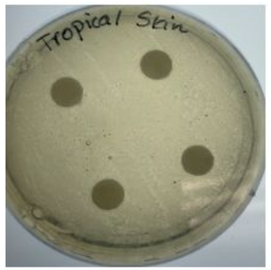
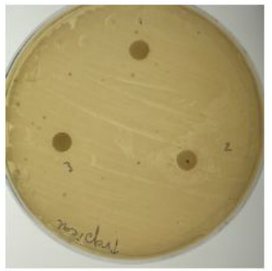
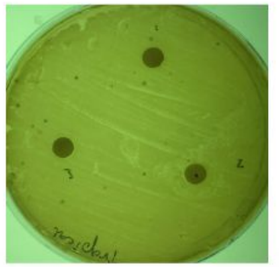
Results Using Bacterial Lawn



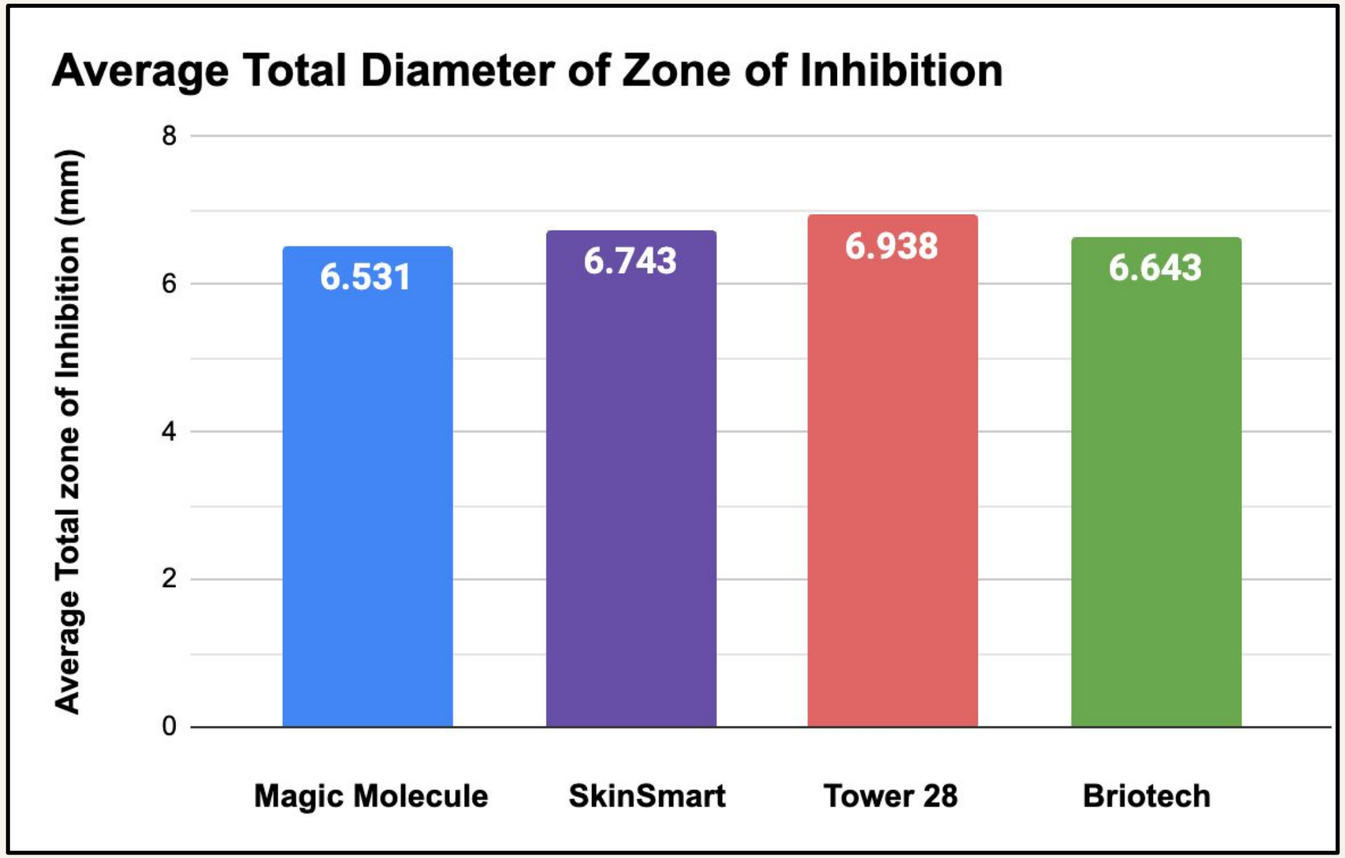
- Magnified Images of clear zones of inhibition from the plates to the right.



- Magnified images of the agar plates displaying the zones of inhibition, that were not perfectly circular.

Brand of Spray	Trail 1 images	Trail 3 (white light)	Trail 3 (green light)
Magic Molecule antimicrobial skin spray			
Skinsmart antimicrobial facial cleanser			
Tower 28 sos daily rescue skin spray			
Briotech Tropical skin spray			

All images contain Mueller-Hinton agar plates with staphylococcus



Results of Spray Brands

The inhibition zones show that the antimicrobial say can kill and reduce the amount of bacteria on the bacteria lawn similarly to how it works on the face meaning that it is effective.

- Tower 28 is the most effective due to having the largest average inhibition zones total at 6.938 mm in diameter.
  - With the diffusion disc being 6 mm and the inhibition zones being measured larger it can be concluded the antimicrobial hypochlorous acid spray can reduce/kill staphylococcus epidermidis cultures.

Antimicrobial Sprays- Images obtained from official brands websites

Discussion and Interpretation

Data and Evidence:

- The **zones of inhibition** around the diffusion discs observed and measured indicate the all four **hypochlorous acid** sprays were able to **reduce growth.**
- The sprays with the highest concentration were not the most effective, Magic Molecule (0.018%) and SkinSmart (0.018%), Tower 28 with (0.015%) concentration was most effective.
- The discs were **6mm**, therefore measuring anything greater than 6mm as shown in Table 1, also confirms this. The diameter of the zones varying slightly, suggests differences in effectiveness due to differences in brands.

Uncontrolled Variables:

- Some zones were not perfectly circular, which may have affected diameter measurements
- Slight differences in disc saturation/ dryness or uneven bacteria lawn could influence results

Effectiveness and Validity:

- Despite not all discs showing zones of inhibition, the observed zones show the ability hypochlorous acid has to inhibit *S. epidermidis* growth.

Reflections and Learning:

- Uniform **bacterial lawns** are crucial for using diffusion discs
- Discs must be placed before bacteria incubation

Conclusions

01

These results supported the first part of my hypothesis that, **Antimicrobial sprays containing hypochlorous acid will inhibit or reduce the growth of Staphylococcus epidermidis,** but not the seconds part, **the spray with the strongest concentration of hypochlorous acid will produce the largest average zones of inhibition.**

- The second part of my hypothesis can not be proved because there is either insufficient data or it is being disapproved by the data found in this experiment
  - **Tower 28 was the most effective brand**

02

Tower 28 SOS spray - Image obtained from Tower 28 website

Final Thoughts

- Using a **bacterial lawn** is essential to produce visual results
- Diffusion discs must be prepared and **air dried** before placement
- Continuing research would help further understand the impact different **concentrations** of hypochlorous acid has on *staphylococcus epidermidis*

Implication and Future research

Since Staphylococcus is one of the most common skin bacteria, understanding how to eliminate its growth with skin safe products could improve bacterial infection problems and personal hygiene (Claudel et al., 2019)

Hypochlorous acid uses for lower recourse locations

- antimicrobial spray being easy to use = accessible way for people with less access to water to avoid facial bacteria and infections.
- suitable for repeated use on areas like the face, skin safe option. HOCl formulations and sprays are also relatively inexpensive to produce, meaning they could be the perfect recourse for settings with less resources (Mendelson et al., 2016).

Future Research

- Continuously adding HOCl to the diffusion disc, daily determine if zones of inhibition become larger (using it frequently inhibits more bacteria)
- Hypochlorous acid (HOCl) is a broad spectrum antimicrobial, highly effective against a wide range of bacteria, to further this research testing this substance on other bacteria (determine if it could be used to inhibit other bacteria growth)
- This research can lead to the development of more effective skincare and improved hygiene that is accessible worldwide

References

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