Determining the effectiveness of alcohol-based sanitizer concentrations on strains of epidermal floras

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Project Objective & Previous Research

A leading U.S. hand sanitizer brand known as Purell jumped 600% in sales directly as a result of the COVID-19 pandemic. Purell kills 99.99% of the most common germs which cause illnesses. 0.01% of bacteria still remains on skin surfaces of the organism. According to Darwin's theory of evolution through natural selection, these bacteria can become immune, or strengthen and develop to grow up to a point where they become super bacteria**. How do common types of epidermal bacteria change in amount/size with the effects of Purell? How effective is Purell? Can bacteria adapt or become immune to the antibacterial substances present in Purell?**

- Alcohol mechanism against bacteria
  - N-propanol is the most commonly used compound in biocides.
    - How does it destroy? Membrane damage, uncoupling of mRNA and protein synthesis through the effects of ribosomes and RNA polymerase (associated with protein protein denaturation)
  - Optimal bactericidal efficacy is at 60-90% concentration, even pure alcohol is less bactericidal (2020).
  - Ethanol/ethyl alcohol > propanol in virucidal activity. Purell is 70% ethyl alcohol/ethanol. Adding aqueous solution to ethanol solutions can increase its efficacy against viruses that are more resistant to ethanol alone. Despite this, it is well known that hand sanitizers are ineffective against non-developed viruses.
- **Resident floras**: ***Staphylococcus epidermidis***, *Bacillus subtilis* (found commonly on hand surfaces) and *Enterococcus faecalis* (not safe for lab use) **colonize deep layers of the skin and are resistant to mechanical removal**.
- **Transient floras: *Escherichia coli B***, *Staphylococcus aureus*,(common cause of respiratory infections, skin infections, etc., pathogenic; colleges and universities only) and *Pseudomonas aeruginosa* (colleges and universities only), *Enterococcus faecium* (very commonly found on hand surfaces/thorough testing done about its effectiveness/growth, pathogenic), **colonize the superficial layers of skin.**
- ABHS are very effective for quickly destroying many pathogens by the action of the aqueous alcohol solution without the need for water or drying with towels (2020).
  - Specific in vitro studies show that hand sanitizers containing 60%-80% ethanol produced 4 to 6 log reductions in 15-30 seconds against a range of bacterial and fungal species (2002).

Hypothesis: Optimal bactericidal efficacy concentrations in destroying harmful epidermal floras.

Materials & Methodology

Procedure

1. Wear gloves and goggles.
2. Take three 50 mL volumetric flasks. Fill with desired concentration amounts.
   a. Low concentration - 45% for 50 mL total solution 22.5 mL of Purell, 27.5 mL of distilled water
   b. Optimal concentration - 70% for 50 mL total solution, 35 mL of Purell, 15 of distilled water
   c. High concentration - 100% for 50 mL total solution 50 mL of Purell, 0 mL of distilled water
   d. Control group - fill with 50 mL of an aqueous solution (distilled water)
3. Using sterile technique and protective equipment, dispense a standard amount of a pure culture of the following types of bacteria, *Escherichia coli B,* a transient flora, and *Staphylococcus epidermidis,* a resident flora into two respective plates.
4. Streak the plate to form a bacterial lawn.
   a. To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in that direction.
   b. Repeat this rotation 3 times to fully swab the plate.
5. Allow the plate to rest for a one to two days in a bacterial incubator to produce starter plates.

Testing - Kirby-Bauer Method

1. Bring both starter plates of the two types of bacteria.
2. Swab into four different respective Petri dishes for *Escherichia coli B* using streaking technique mentioned above. Label with bacterial name and present date.
3. Insert 250 microliters in the middle of the Petri dish for each of the four concentration types, respectively. Label with concentration type. (Optional: for next test, apply in 5 distinct areas of the Petri dish to observe consistency.)
4. Repeat testing steps to create dishes for *Staphylococcus epidermidis.*
5. Incubated all 8 plates over three days at a temperature of 37 °C (98.6 °F).
6. Record zone of inhibition and observe antimicrobial efficacy.

Experimental group: ABHS (alcohol-based hand sanitizer) concentration solution, Control group: NABHS (non-alcohol based hand sanitizer) concentrated solution/aqueous

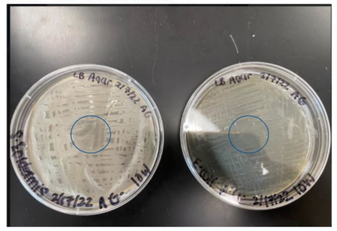
Abstract

Purell kills 99.99% of the most common germs which cause illnesses. This means that 0.01% of bacteria still remains on the skin surfaces of the organism. Optimal bactericidal efficacy is at 60-90% concentration as even pure alcohol is less bactericidal. Purell consists of ethanol which proves to be most effective in virucidal activity. Adding aqueous solution to ethanol solutions can increase its efficacy against viruses that are more resistant to ethanol alone. However, it is well known that hand sanitizers are ineffective against non-developed viruses. Alcohol-based hand sanitizers are very effective for quickly destroying many pathogens through aqueous alcohol solutions. This study tests the effectiveness of alcohol-based sanitizer concentrations on specific types of epidermal bacteria. This experiments alcohol-based sanitizer concentrations on two types of epidermal floras, Staphylococcus epidermidis and Escherichia coli B. To test this hypothesis, a two-part procedure was conducted using the Kirby-Bauer method. Low, high, and optimal concentration solutions are formulated as the experimental group while the control group is a pure aqueous solution. Using sterile technique, cultures of Staphylococcus epidermidis and Escherichia coli B were dispensed onto Petri dishes and tested with each different concentration type. The results of Escherichia coli B disproved the hypothesis as high concentration levels were most effective in regulating its presence while Staphylococcus epidermidis modulated best under optimal concentrations. This considers the effectiveness of sanitizer on bacteria that remains on the epidermis even after Purell is applied and can display how concentration modifications affect the growth of bacteria.

Results

Test 1 (2/15 - 2/17) - 250 microliters (1)

Low Concentration
S.Epidermis - 0.6 cm in diameter
E.coli - 0.45 cm in diameter



Optimal Concentration
S.epidermis - 0.5 centimeters in diameter
E-coli B - 0.3 centiments in diameter

Figure 4 and 5: Zone of inhibitions for two types of bacterial strains in contact with a low and optimal concentration of sanitizer, respectively.

High Concentration
S.epidermis - 0.7 centimeters in diameter
E-coli B - 0.25 sentiments in diameter

Control Group
No zone of inhibhition for *S.epidermis* or *E.coli*

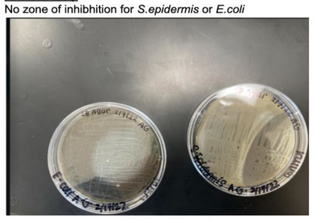
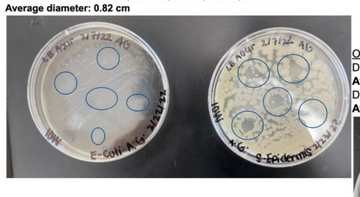


Figure 6 and 7: Zone of inhibitions for two types of bacterial strains in contact with a high concentration of sanitizer and the control group concentration, respectively.

Test 2 (2/23 - 2/25) - 250 micrometers (5)

Low Concentration
Diameters for *S.epidermis*: 0.7 cm, 1.5 cm, 0.75 cm, 0.55 cm, 0.45 cm
Average diameter: 0.79 cm
Diameters for *E-coli*: 0.8 cm, 0.65 cm, 0.9 cm, 0.8 cm, 0.95 cm
Average diameter: 0.82 cm



Optimal Concentration
Diameters for *S.epidermis*: 1.1 cm, 0.7 cm, 1.25 cm, 0.9 cm, 0.75 cm
Average diameter: 0.94 cm
Diameters for *E-coli*: 0.7 cm, 1.2 cm, 0.95 cm, 2.2 cm, 1.75 cm
Average diameter: 1.36 cm

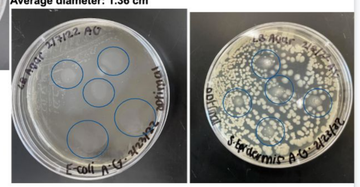
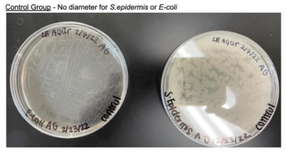


Figure 8 and 9: Zone of inhibitions for two types of bacterial strains in contact with a low and optimal concentration of sanitizer, respectively.

High Concentration
Diameters for *S.Epidermis*, respectively: 0.85 cm, 0.9 cm, 0.7 cm, 0.8 cm, 0.75 cm
Average diameter: 0.8 cm
Diameters for *E-coli*, respectively: 1.6 cm, 1.5 cm, 2.5 cm, 1.6 cm, 1.4 cm
Average diameter: 1.72 cm

Figure 10 and 11: Zone of inhibitions for two types of bacterial strains in contact with a high concentration of sanitizer and the control group concentration, respectively.

Control Group - No diameter for *S.epidermis* or *E-coli*



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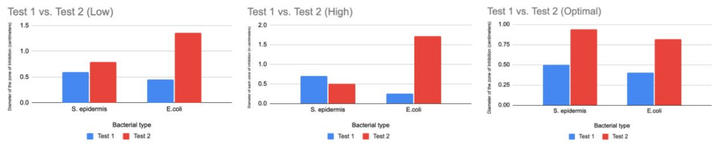
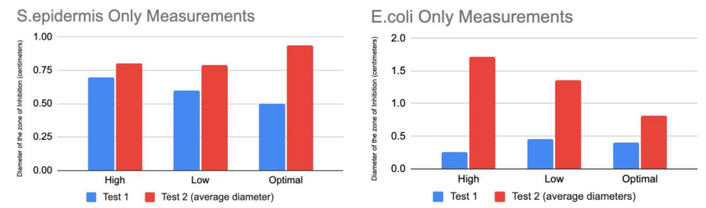


Figure 12-16: Graphical representations of results



Results & Interpretation

- In both tests, *S.epidermidis* was consistent in showing efficacy as the zone of inhibition remained higher than *E.coli* in almost every single test.
- The optimal solution is intended to be a 70% concentration which proves to be most effective, as concluded by a 2002 study however it only proved to do so for the *S.epidermidis* culture and was inconsistent with the *E.coli.*
- None of the control groups presented any zone of inhibition even thought here was the aqueous solution displaced in both tests.
- Each dish resulted in distinct zone of inhibitions, indicating that Purell is effective.
  - In general, it proved to be more effective on *S.epidermidis* rather than *E.coli.* The expectation was the other way around as *E.coli* is the transient flora when observing what kind of bacteria still remains after sanitizer use while *S.epidermis* is a residential epidermal flora.
- Possible Errors
  - After conducting the first test, it was evident that multiple insertions are needed on the Petri dish to be able to quantify growth. The second test ended up being five distinct displacements of concentration solutions while the first test was only one per dish. Due to time constraints, I was not able to repeat the experiment a third time to observe the regularity of five insertions instead of one in the middle.This may have resulted in a discrepancy when measuring the average diameters of the zone of inhibitions.
  - Midway through the experiment, I realized that the concentrations can be done with various other alcohol types to test which is more efficient as well (ex. aloe vera, rubbing alcohol, n-propanol vs. isopropanol, etc.)

Conclusion

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Figure 17: *Escherichia coli B*

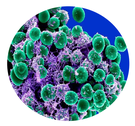

Figure 18: *Staphylococcus epidermidis*