The Antibacterial Properties of Manuka Honey on the Gram Negative Bacteria, *Providencia stuartii* 

Wanda Scott  
199400147  
April 1, 2013  

Wanda Scott  wscott@mhc.ab.ca  
Tristyn Carpenter  tristyn.carpenter@mymhc.ca  
Monica Schafer  monica.schafer@mymhc.ca  
Ashley Moss  ashley.moss@mymhc.ca
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Part One

Identification of Unknown Bacteria

Streak plate from soil broth solution and tested for identification.

Data

Bacteria obtained from soil samples was isolated and colonized for identification. Gram staining was done in the usual method and purple bacillus shape confirmed. Catalase confirmed with water bubbles produced.

<table>
<thead>
<tr>
<th>Test Done</th>
<th>Result</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol Red Carbohydrate Media</td>
<td>Pink at top/yellow</td>
<td>anaerobic</td>
</tr>
<tr>
<td>Phenol Red Broth</td>
<td>yellow/orange/positive</td>
<td>acid and fermentation utilizable glucose or lactose</td>
</tr>
<tr>
<td>Methyl Red Voges Proskauer</td>
<td>yellow/negative</td>
<td>acetic, lactic and succ small acid formation</td>
</tr>
<tr>
<td>Organic Acid Metabolism</td>
<td>green</td>
<td>cannot utilize citrate</td>
</tr>
<tr>
<td>Urease</td>
<td>pink</td>
<td>positive for ammonia and C02</td>
</tr>
<tr>
<td>Catalyse</td>
<td>bubbles</td>
<td>positive for H2O formation</td>
</tr>
<tr>
<td>Test Done</td>
<td>Result</td>
<td>Explanation</td>
</tr>
<tr>
<td>------------</td>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Gram Stain</td>
<td>Red, pill shaped, unclustered</td>
<td>gram negative bacilli bacilli</td>
</tr>
<tr>
<td>Motility</td>
<td>slight movement</td>
<td>positive</td>
</tr>
</tbody>
</table>
Gram staining

cocci                   bacillis

red

Phenol red +        Non-pigmented colonies

Phenol red lactose       Phenol red lactose -

Urease +       Motility +       Trypt +

Providencia rettgeri
*Providencia rettgeri*

This was originally assigned to the genus *Shigella* in 1927, but was removed after it was found to have produced indole and was motile. In 1943 it was moved to the genus *Protus*, and finally resides in *Providencia* group since 1978. It is found in solid waste, soil and marine environments.

*Providencia rettgeri* has interacted with loggerhead turtles, human, insects and frogs. (Penner et al., 1979)

*Providencia rettgeri* can be found in human gut, stool and is a common cause of urinary tract infections. "It may also be responsible for cause of keratitis, dacyocystitis or conjuntiitus" (Koreishi et al., 2006). It has also been the cause of *travelers diarrhea*.

Morphology reveals short rod shaped, non-motile and are white colonies on a agar plate. Treatment is effective with..." aminoglycosides, quinolones, fosformycin and B-lactam based antibiotics." (Stock and Wiedemann, 1998)

Suggested uses for this bacteria is to perform "biotransformation off certain heavy metals such as aluminum, cobalt and copper." (Education Lifedesk, 2013). It may replace the two step ammonia from wastewater. (Zhao et al., 2010). Lastly it is a major producer of penicllin G amidase. (Education Lifedesk, 2013)
Part Two

The Antibacterial property of Manuka honey on *Providencia stuartii*

**Introduction**

Honey is manufactured from honey bees from the "collection of nectar or blossom or the collection of the secretions ...or excretions from plants", (Jawad, 2011). Honey has been used to fight infection as an antimicrobial and wound healing properties since ancient times (Kwakman et al., 2008). Now honey is making a come back in modern medicine, as a antimicrobial agent, treatment of burns, postoperative wounds and even the treatment of wounds from Methicillin-resistant *Staphylococcus aureaus*. It is also noted by Jawad to speed the growth of new tissue and is bactericidal and bacteriostatic against both gram negative and positive.

Sabastian A. J. Zaat experimented with medical grade honey and antibiotic resistant bacteria. He isolated the defensin-1 protein which is added from bees. This is the protein compound is responsible for the antibacterial property found in honey (Zaat et al., 2010). Others believe that high sugar content, the production of hydrogen peroxide, and a naturally low pH (Zaat et. al., 2011), all play a role in the antibacterial properties. "A compound methylglyoxal (MG) a reactive precursor in the formation of advanced glycation end products", Adams el. a., 2008). Other there seems to be some discussion on exactly which of the properties contribute to and positive effects on bacterial growth, all acknowledge the benefit.
Worth exploring is the synergistic effect of both antibiotics and medicinal honey.

According to Jawad (2011), "The mean inhibition zone produced by honey when applied to isolated gram-negative bacteria (Psedomonas aeruginosa) and isolated gram-positive bacteria (coagulase positive Staphylococci) was significantly higher than that of the antibiotic used...and when combination of both honey and antibiotic was used, it showed highly significant increased sensitivity than that of honey or antibiotic alone".

Bacitracin is a broad spectrum antibiotic and typically used as a topical preparation, although only prescribed for Staphylococcal. The mechanism of action for bacitracin is the suppression of protein synthesis. It has been known to produce, neurogenic and renal toxicity and has been part of the problem with the antibiotic resistance. The name derived from the first isolation in 1943, produced from a "licheniformis group of Bacillus subtilis var Tracy ...from a knee scrape from a girl named Margret Tracy." (Drug Bank, 2013)

Kanamycin is an antibiotic used for the treatment of both gram positive and gram negative bacteria, although a greater effect in the negative. The destruction of the bacteria is accomplished by binding to the 30S ribosomal subunit of the t-RNA, leading to misreading. This aminoglycoside antibiotic makes the bacterium unable to synthesise proteins. "Kanamycin in used for anaerobic bacteria, fungi and viruses." (Drug Bank, 2013) Best used synergistically with other antibiotics for treating streptococcal infections. The side effects are accumulation in the renal tubules and may cause reversible nephrotoxicity in 5 - 25%. (Drug Bank, 2013)
Hypothesis

The purpose of this experiment is to determine the natural antibacterial properties of Manuka honey by measuring the zone of inhibition. To determine a synergistic effect of honey and antibiotic, both will be combined to our bacterial plate and zones measured. The predicted outcome is that the Manuka honey will equal or surpass Kanamycin and Bacitracin. The effect of both an antibiotic and honey should also surpass either alone.

Materials

• Masking Tape
• Marker
• Forceps
• Inoculating Loop
• Ruler
• Pipette
• 6 discs of Kanamycin
• 6 discs of Bacitracin
• Incubator
• 4 Glass Slides
• Manuka Honey
• Gram Stain Reagents
• Drinking Straw

• Bunsen Burner

• 2 Test Tubes of Nutrient Broth

• Vortex

• 18 Mueller – Hinton Agar Plates

• Bacteria from Soil Sample on Streak Plate

• Spreader

• Alcohol Solution

• Streak Plate of Enterobacter aerogenes

• Streak Plate of Staphlococcus epidermidis

• Distilled Water

• Electronic Scale

• Metal Scoop

• Microscope

**Procedure**

1. Prepare bacterial lawns by using inoculating loop, after flaming it, to pick up an isolated colony of the bacteria from a streak plate. Flame the top of test tube with nutrient broth and put inoculating loop with bacteria in broth and swirl around inside to deposit the bacteria. Flame top of test tube and inoculating loop and mix test tube with broth in vortex. Repeat the above steps
with new materials and the E. aerogenes bacterium, to create a bacterial broth for it. Repeat this step as well for the S. epidermidis to create its bacterial broth.

2. Pipette 0.1mL of bacterial broth, *Providencia rettgeri* to the agar plate and use spreader, which has been flamed, to ensure bacteria are dispersed across plate. Flame spreader between each agar plate.

3. Do the second step for all agar plates, flaming the spreader between each. On 3 of the agar plates pipette both 0.10 mL of the soil bacterium broth on to plate, as well as 0.10 mL of E. aerogenes bacterial broth and 0.10 mL of the S. epidermidis bacterial broth. Repeat rest of steps. Label and incubate the 3 agar plates with the soil bacterium, E. aerogenes and S. epidermidis for 48hrs at 37 degrees Celsius.

4. Let 6 of the agar plates dry for approximately five minutes. On 3 of the agar plates with forceps place 1 antibiotic disc of Kanamycin centred in the middle of plate, repeat this on 2 more agar plates flaming the forceps between each plate. Lightly touch each disc with your sterile inoculating loop to make sure that it is in good contact with the agar surface. On the other 3 agar plates repeat this step with the antibiotic discs of Bacitracin. Label all six agar plates appropriately and place in incubator at 37 degrees Celsius for 48hrs.

5. On 3 agar plates use the drinking straw to punch make a hole in the agar. Using the electronic scale measure out 1g of Manuka honey for each agar plate. Place the honey in the hole by using metal scoop. Make sure that honey does not overflow the hole. Label agar plates accordingly and incubate at 37 degrees Celsius for 48hrs.
6. On the remaining 6 agar plates make a hole with the drinking straw to create well. Fill hole half way with the Manuka honey. In 3 of the agar plates place a Bacitracin antibiotic disc in the half filled hole; in the other 3 use Kanamycin antibiotic discs. Proceed to fill the rest of the well with honey and incubate at 37 degrees Celsius for 48hrs.

7. Remove all agar plates from incubator and record in data tables your observations and the size of the zone of inhibition. Photograph each plate to look back on as a reference and for report observations.

8) Observations will be recorded in data tables, pictures and a line graph that shows the size of the zone of inhibition with the different tests. There will also be pictures of microscope slides to show more observable effects of the antibiotics, honey and bacteria. The statistics we will use will be the size of the zone of inhibition, and the data tables will record the visible observations and any zone of inhibition that is present.

Results

The plate smear with *Providencia rettgeri* was smeared with both *E. aerogenes* and *S. epidermidis* to determine any antibacterial properties. No zones on inhibition were found. This led to a negative finding.

Result findings for Manuka honey showed interesting results. Not only was there a significant zone of inhibition, an average of 0.9 cm. There was a secondary zone with limited inhibition that measured an average of 2.13 cm. The zone of secondary inhibition was not complete eradication, but the area far exceeded the antibiotic zone of inhibition alone.
The zones of inhibition of the combined antibiotic Kanamycin and honey did indeed show a synergistic effect. Major zone averages were slightly lower combined (1.15 cm), than with antibiotic alone (1.28 cm), but the secondary partial inhibition zone was completely void in the control Kanamycin, but showed gains with the combined of 1.7 cm average.

The control agar disks inoculated with Bacteracin disks had no zones of inhibition. The combined honey with disks averaged 0.75 cm, but again the agar had a secondary zone of partial inhibition measuring average of 1.75cm.

### Primary Zone of Inhibition

<table>
<thead>
<tr>
<th>Primary Zone</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuka Honey</td>
<td>0.9</td>
<td>0.95</td>
<td>0.85</td>
</tr>
<tr>
<td>Manuka Honey and Bacteracin</td>
<td>0.75</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Manuka Honey and Kanamycin</td>
<td>1.25</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

![Graph showing zones of inhibition](image-url)
## Secondary Zone of Inhibition

<table>
<thead>
<tr>
<th>Secondary Zone</th>
<th>2nd Zone</th>
<th>1st Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuka Honey</td>
<td>2.15 cm</td>
<td>2.5 cm</td>
</tr>
<tr>
<td>Manuka and Bactiracin</td>
<td>1.80 cm</td>
<td>1.55 cm</td>
</tr>
<tr>
<td>Manuka and Kanamycin</td>
<td>1.7 cm</td>
<td>1.8 cm</td>
</tr>
</tbody>
</table>

## Comparative Zones of Inhibition in Antibiotics and Manuka Honey

### Bar Chart

- **Honey**: 2nd Zone: 2.25 cm, 1st Zone: 1.5 cm, 2nd Zone: 0.75 cm
- **Bactiracin**: 2nd Zone: 1.8 cm, 1st Zone: 1.5 cm
- **Kanamycin**: 2nd Zone: 1.5 cm, 1st Zone: 0.75 cm
Errors

Difficulties with this experimental model were in the measurement of honey. Due to the viscosity, varying amounts were applied to the agar holes. Plates were incubated and then refrigerated for 6 days until the results could be read, which could have caused growth changes. Errors could have been made in the measurement of the zones, using ruler and eyesight.

Conclusion

Manuka honey did indeed show a definite zone of inhibition on the bacteria *Providencia rettgeri*. The secondary zone was unexpected and not reviewed in any literature, but showed exciting new changes for the use of honey medicinally. The secondary zone proved the synergy with Kanamycin. This shows great promise for the use topically, but possibly internally as well.
References


Jawad, R., A., H., (2011). Antimicrobial effect of bee honey on some pathogenic bacterial isolated from infected wounds in comparison to commonly used antibiotics. *Journal of Basrah Researches (Sciences)* Volume 37, Number 4

