Exploration of Texas Honey as a Treatment for Strep throat Caused by Streptococcus pyogenes

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Abstract
Streptococcus pyogenes (S. pyogenes) commonly causes strep throat. Traditional treatments involve antibiotics or tonsil removal, but this raises concerns about antibiotic resistance and immune system suppression. Recent research indicates S. pyogenes can internalize, reducing external antibiotic efficacy. Consequently, honey emerges as a potential alternative treatment due to its complex chemical composition and antibacterial properties. Recent studies show that several honey species inhibit S. pyogenes, including antibiotic-resistant strains. This study assessed various Texas honeys’ antibacterial and antimicrobial effectiveness against S. pyogenes through agar optimization, zone of inhibition (ZOI), and XTT assays. S. pyogenes grew well on Brain Heart Infusion (BHI) agar in a CO2-rich environment. Honey samples inhibited bacterial growth, with 23H-32 showing the greatest ZOI. Seven samples exhibited antimicrobial activity above 50%, with 23H-32 having the highest XTT value of 77.24%. A direct, positive relationship between ZOI and XTT was observed, suggesting 23H-32 as a potential treatment option. Further investigation into honey’s physicochemical properties, like minimum inhibitory concentration (MIC) and hydrogen peroxide (H2O2) levels, is needed to understand variations among honey samples.

Introduction
-Streptococcus pyogenes (S. pyogenes), known as Group A Streptococcus (GAS), is a prominent human pathogen responsible for severe infections globally. Clinical manifestations of GAS infections include strep throat, characterized by sore throat, fever, and red and swollen tonsils. Treatment modalities for GAS infections often involve antibiotic therapy and, in some cases, surgical intervention such as tonsillectomy. The increasing prevalence of antibiotic usage contributes to the emergence of antibiotic-resistant bacteria, complicating treatment efficacy. Further, complications arise through side effects, drug interactions, and drug resistance. The severity of strep throat varies from mild to severe, with strep infections leading to complications like rheumatic fever, nephritic syndrome, and acute glomerulonephritis. Approximately 33% of people who develop strep throat experience recurrences, and 8% continue to experience symptoms, including fever and vomiting.

Methods
- Cultivation of bacteria: S. pyogenes was diluted to McFarland Standard 0.5 MFU, and cultivated on Mueller Hinton (MH) Brain Heart Infusion (BHI), and Chocolate agar. Three samples were placed in the inoculator, and another three were plated in a candle jar and incubated for 24 hrs at 37°C.
- Well Diffusion Assay: The diluted bacteria sample was plated on BHI agar, and studies demonstrate plate morphology on each plate. The honey samples were added to wells four times, and dH2O was added to one as a negative control. Pencillin-G (200 µg/mL) was used as a positive control. The samples were incubated for 24 hrs at 37°C, and the ZOI was measured.
- XTT Colorimetry: An XTT-Membrane working solution was prepared on the day of the analysis. The bacterial sample was further diluted to 1/10 in BHI, and the honey samples to 1/2. The honey samples, BHI broth (negative), Pencillin-G (positive), BHI broth (positive), and dH2O (blank) were added to a 96-well plate. The bacterial sample was added to all wells except the blanks. The well plate was incubated for 24 hrs at 37.5°C, 5% CO2 with a 1% shaking rate at 75 rpm. After incubation, the solution was added and incubated for 20 minutes at 37°C in the shaker incubator (75 rpm). After incubation, the absorbance was read at 490 nm using a microplate reader.

Results
-Growth of S. pyogenes on Different Agars and Environments

<table>
<thead>
<tr>
<th>Sample</th>
<th>Environmental Condition</th>
<th>Zone of Inhibition (ZOI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23H-19</td>
<td>Incubated 37°C</td>
<td>16</td>
</tr>
<tr>
<td>23H-20</td>
<td>Incubated 37°C</td>
<td>20</td>
</tr>
<tr>
<td>23H-32</td>
<td>Incubated 37°C</td>
<td>24</td>
</tr>
<tr>
<td>23H-35</td>
<td>Incubated 37°C</td>
<td>28</td>
</tr>
<tr>
<td>23H-42</td>
<td>Incubated 37°C</td>
<td>30</td>
</tr>
<tr>
<td>23H-45</td>
<td>Incubated 37°C</td>
<td>32</td>
</tr>
<tr>
<td>23H-48</td>
<td>Incubated 37°C</td>
<td>35</td>
</tr>
</tbody>
</table>

Conclusion
-Agar and Environmental Optimization for Streptococcus pyogenes

-BHI medium contained the greatest growth of bacteria after 24 hours, and the greatest sensitivity to treatments presented on BHI agar and placed in a candle jar then incubated at 37°C for 24 hours.
-Brain Heart Infusion (BHI) was to be used as the medium to grow the bacteria, and the final results were tested in the collection.

Zone of Inhibition of Texas Honey Samples against Strep throat Caused by Streptococcus pyogenes

- Honey samples 23H-19, 26, 32, 35, 40, 42, 46, 48, 49 were the top ten honey samples on the greatest ZOI.
- Honey sample 23H-45 had the greatest overall ZOI, thus it was estimated that the top ten honey samples specifically 23H-45 would have high antibacterial activity in relation to S. pyogenes.
- In comparison to positive control, the top ten honey samples were within a reasonable range of the antibiotic concluding the honey’s antibacterial activity levels were of high variety.

- XTT assay of ten Texas honey samples with greatest ZOI/antibacterial activity
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References

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