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 compromise. 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 demonstrate honey's effectiveness against certain pathogens, 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 best on Brain Heart Infusion (BHI) agar in a CO2-rich environment. Honey samples inhibited bacterial growth, with 23H-32 showing the largest 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. However, 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[4]. Treatment modalities for GAS infections typically 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 strains, complicating treatment efficacy[10].

-Regarding complications with Strep Throat treatments, honey emerges as a promising alternative therapy. Honey has a complex chemical composition that varies due to botanical source[5]. Chemically, honey contains a complex blend of many inorganic and organic compounds. Generally, honey comprises carbohydrates, water, proteins, amino-acids, phenols, enzymes, and polyphenols[2]. These constituents exhibit significant antibacterial and antimicrobial properties, attributed to osmotic effects, pH modulation, and hydrogen peroxide generation[3].

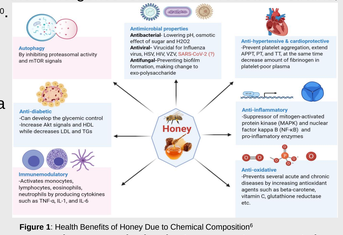

Figure 1: Health Benefits of Honey Due to Chemical Composition[6]

-Studies have demonstrated the efficacy of honey, against various pathogens, including antibiotic-resistant strains like methicillin-resistant Staphylococcus aureus (MRSA)[7,8]. Furthermore, honey's antioxidant, anti-inflammatory, and anti-cancer properties, coupled with its hygroscopic nature, render it capable of inhibiting microbial growth and virulence, particularly against S. pyogenes infections[1-9]. Despite these promising findings, the precise scale of honey's effectiveness against strep throat caused by S. pyogenes remains unclear.

Purpose

This study aimed to explore various Texas honey samples' antibacterial and antimicrobial abilities against *S. pyogenes*. This was utilized through agar optimization, zone of inhibition, and XTT assays. Therefore, if Texas honey samples are grown in an optimized medium and tested against *S. pyogenes*, it is expected that the honeys will show an ability to prevent growth and eliminate the bacteria due to honeys antibacterial and antimicrobial properties. Through this investigation, Texas honey samples found with superior antibacterial and antimicrobial properties against *S. pyogenes* could serve as viable alternatives for patients with antibiotic-resistant or recurrent strep throat infections.

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 incubator directly, and another three were placed 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 five wells were made on each plate. The honey samples were added to four wells, and dH2O was added to one as a negative control. Penicillin-G (200 ug/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-Menadione 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), Penicillin-G (positive), BHI broth only (blank), 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 35.5°C in a shaker incubator at 75 rpm. After incubation, the working solution was added and incubated again for 30 mins at 35.5C 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

Agar	Environment	Description of Growth after 24 Hr
BHI Agar	Incubator 37°C	Thin layer of bacteria was present on entire plate, bacteria was transparent with slight cloudiness
MH Agar	Incubator 37°C in	Bacteria growth was unclear, very small colonies observed on plate only seen at some angles of light
Chocolate Agar	Incubator 37°C in	Thin layer of bacteria was present on plate, bacteria fully transparent
BHI Agar	Incubator 37°C in Candle Jar	Thick layer of bacteria was present on entire plate, bacteria was very cloudy with many colonies of strep.
MH Agar	Incubator 37°C in Candle Jar	Bacteria growth was unclear, small colonies observed on plate only seen at some angles of light
Chocolate Agar	Incubator 37°C in Candle Jar	Thin layer of bacteria was present on entire plate, bacteria growth was greater than without candle jar.

Table 1. Bacteria growth on agar demonstrated the ability of *S. pyogenes* to grow on BHI, MH, and Chocolate Agar in two different environments. One environment was an incubator at 37°C and the second environment was a candle jar in an incubator at 37°C which was used to increase the CO_2 levels. The range of bacteria growth was present as no bacteria grown, thin layers of bacteria, and thick layers with colonies present. BHI agar in candle jar placed in an incubator at 37°C displayed the most *S. pyogenes* growth.

Texas Honey Sample ZOI on *S. pyogenes*

Sample	ZOI (mm)
Penicillin 1	25.85
23H-01	15.49
23H-02	18.42
23H-03	15.22
23H-04	18.30
23H-05	15.14
23H-11	12.94
23H-13	15.79
23H-14	12.42
23H-15	18.35
23H-16	17.33

Sample	ZOI (mm)
Penicillin 2	26.35
23H-17	18.67
23H-18	17.59
23H-19	20.91
23H-20	17.41
23H-21	19.86
23H-23	17.43
23H-24	15.05
23H-25	16.68
23H-26	24.50
23H-27	16.12

Sample	ZOI (mm)
23H-28	17.57
23H-31	20.12
23H-32	25.01
23H-33	20.38
23H-34	20.37
23H-35	23.66
23H-36	17.50
23H-37	15.34
23H-38	19.98
23H-39	15.62

Sample	ZOI (mm)
23H-40	22.81
23H-41	20.34
23H-42	20.92
23H-43	19.79
23H-44	16.09
23H-45	20.53
23H-46	25.03
23H-47	19.31
23H-48	22.74
23H-49	23.41

Table 2. The ZOI data enabled the honey to be analyzed as having either high antibacterial activity or low antibacterial activity against *S. pyogenes* when plated on BHI agar and placed in a candle jar in an incubator at 37°C for 24 hours. The collected ZOI for Texas honey samples ranged from 12.42 mm to 25.03 mm with no variation. The higher antibacterial activity honeys were within a range of 20.53 mm to 25.03 mm. The positive control Penicillin had ZOIs ranging from 25.85-26.35 mm which corresponded with great antibacterial activity.

Results - con't

ZOI of Select Texas Honey Samples

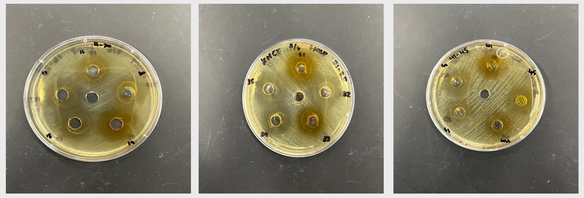

Figure 2. Texas honey samples were plated in wells on *S. pyogenes* cultivated on BHI agar. The honey samples depicted are 16-20, 31-35, and 41-45. The honey samples show inhibition of bacterial growth.

XTT Colorimetry of Texas Honey Samples with Top 10 ZOI

Sample ID	XTT (%)	ZOI (mm)
Penicillin G in BHI	97.59	26.35
23H-19	51.72	20.91
23H-26	0.00	24.50
23H-32	77.24	25.01
23H-35	67.38	23.66
23H-40	44.86	22.81
23H-42	69.79	20.92
23H-45	64.38	20.53
23H-46	68.72	25.03
23H-48	52.97	22.74
23H-49	9.66	23.41

Table 3. XTT data allowed honeys to be broadly quantified as to having either relativity high cellular proliferation/antimicrobial activity or relativity low cellular proliferation/antimicrobial activity against *S. pyogenes*. The higher antimicrobial honeys proliferated the *S. pyogenes* resulting in a lower absorbance reading and high XTT reading, while the lower antimicrobial honeys proliferated less *S. pyogenes* resulting in a higher absorbance reading and low XTT reading. The XTT for Texas honey samples ranged from 0%-77.24%.

Comparison of Texas honey samples XTT and ZOI

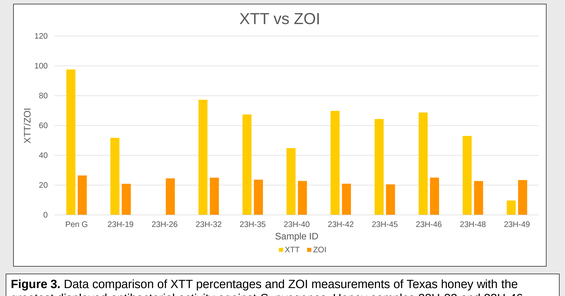

Figure 3. Data comparison of XTT percentages and ZOI measurements of Texas honey with the greatest displayed antibacterial activity against *S. pyogenes*. Honey samples 23H-32 and 23H-46 displayed the greatest XTT and ZOI against *S. pyogenes*. The overall trend on data collection for XTT and ZOI of Texas Honeys observed is proportional.

Conclusions

- **Agar and Environmental Optimization for *Streptococcus pyogenes***
  - BHI medium contained the greatest growth of bacteria after 24 hours, and even further growth was present after plated 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 *S. pyogenes,* in later trials, as demonstrated in the collected data.
- **Zone of Inhibition of Texas Honey Samples against *Streptococcus pyogenes***
  - Honey samples 23H-19, 26, 32, 35, 40, 42, 45, 46, 48, 49 were the top ten honeys with the greatest ZOI
  - Honey sample 23H-45 had the overall greatest 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 reasonable range of the antibiotic concluding the honeys' antibacterial activity levels were of high variety.
- **XTT assay of ten Texas honey samples with greatest ZOI/antibacterial activity**
  - Honey samples 23H-32 and 23H-46 had the overall highest XTT values at 77.24 and 68.72, respectfully.
  - The high XTT values for sample 23H-32 and 23H-46 in relation to the positive control were determined within range of one another thus it was estimated that 23H-32 and 23H-46 would have high antimicrobial activity, cellular proliferation, viability, and cytotoxicity against *S. pyogenes.*
- **Comparison of Texas honey samples XTT and ZOI**
  - Honey samples 23H-32 and 23H-46 had the highest XTT and ZOI, thus supporting the honey samples had the greatest antibacterial and antimicrobial activity against *S. pyogenes* in relation to the Texas honeys.
  - The correlation between ZOI and XTT was determined to be directly related.
- Overall, this study underscores the potential of certain Texas honeys, particularly 23H-32 and 23H-46, as effective antimicrobial agents against *S. pyogenes.* Due to Texas honey samples 23H-32 and 23H-46 effective antimicrobial activity against *S. pyogenes* it can further be investigated the use of honey as a treatment for strep throat in patients with antibiotic resistance and patients with reoccurring strep throat.

References

[1] Al-Waili NS, Haq A. Effect of honey on antibody production against thymus-dependent and thymus-independent antigens in primary and secondary immune responses. *J Med Food.* 2004;7(4):491-494. doi:10.1089/jmf.2004.7.491
[2] Bhargav HS, Shastri SD, Poornav SP, Darshan KM, Nayak MM. Measurement of the Zone of Inhibition of an Antibiotic. Paper presented at: 2016 *IEEE 6th International Conference on Advanced Computing (IACC).* 2016 Feb 27-28; Bhimavaram, India, doi: 10.1109/IACC.2016.82.
[3] Chhawchharia A, Haines RR, Green KJ, Barnett TC, Bowen AC, Hammer KA. In vitro antibacterial activity of Western Australian honeys, and manuka honey, against bacteria implicated in impetigo. *Complement Ther Clin Pract.* 2022;49:101640. doi:10.1016/j.ctcp.2022.101640
[4] Ebell MH, Smith MA, Barry HC, Ives K, Carey M. The rational clinical examination. Does this patient have strep throat?. *JAMA.* 2000;284(22):2912-2918. doi:10.1001/jama.284.22.2912
[5] Eteraf-Oskouei T, Najafi M. Traditional and modern uses of natural honey in human diseases: a review. *Iran J Basic Med Sci.* 2013;16(6):731-742.
[6]Hossain KS, Hossain MdG, Moni A, et al. Prospects of honey in fighting against COVID-19: pharmacological insights and therapeutic promises. *Heliyon.* 2020;6(12):e05798. doi:https://doi.org/10.1016/j.heliyon.2020.e05798
[7] Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed.* 2011;1(2):154-160. doi: 10.1016/S2221-1691(11)60016-6
[8] Miguel MG, Antunes MD, Faleiro ML. Honey as a Complementary Medicine. *Integr Med Insights.* 2017;12. doi:10.1177/1178633717702869
[9] Nassar HM, Li M, Gregory RL. Effect of honey on Streptococcus mutans growth and biofilm formation. *Appl Environ Microbiol.* 2012;78(2):536-540. doi:10.1128/AEM.05538-11(
[10] Shulman ST, Bisno AL, Clegg HW, et al. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America [published correction appears in Clin Infect Dis. 2014 May;58(10):1496. Dosage error in article text]. *Clin Infect Dis.* 2012;55(10):1279-1282. doi:10.1093/cid/cis847

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