Antimicrobial Activity of Honeys Against ESKAPE and *F. novicida* Mutants

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Abstract

Honey has long been recognized for its broad-spectrum antimicrobial properties, attributed to a combination of physicochemical characteristics and bioactive compounds. Its effectiveness against both Gram-positive and Gram-negative bacteria positions it as a promising natural agent for infection control. However, antimicrobial efficacy can vary significantly across different honey types. In this study, we evaluated the antibacterial activity of five distinct honey samples—including Manuka, SA13, U4, NJ15, and G2—against key bacterial species from the ESKAPE group, as well as *Francisella novicida* wild-type and selected metabolic mutants. Using zone of inhibition assays, we observed measurable antimicrobial effects across all tested honeys against the target organisms. Furthermore, we investigated the impact of honey on *F. novicida* as an environmental model organism and extended our analysis to various *F. novicida* mutants to identify specific genetic and metabolic pathways influenced by honey treatment. These findings provide insights into the differential bioactivity of honey and its potential mechanism of action at the molecular level.

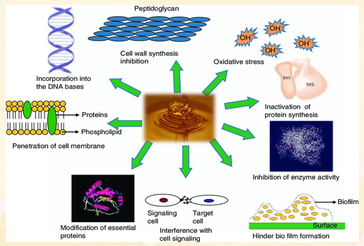
Introduction

Honey is a natural substance produced by honeybees from the nectar or honeydew through a complex enzymatic process. It contains over 200 bioactive compounds, including sugars, amino acids, vitamins, minerals, enzymes, flavonoids, phenolic acids, and antioxidants. Among its many health benefits, **honey's antimicrobial activity** has gained increasing attention in the face of rising antimicrobial resistance (AMR)—a major global health concern that compromises the effectiveness of existing antibiotics.

The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*) are particularly notable for their ability to "escape" the effects of multiple antibiotics. These multidrug-resistant organisms are commonly associated with healthcare-acquired infections and can form biofilms, making them especially difficult to treat. Their growing resistance underscores the urgent need for alternative antimicrobial therapies.

In this study, we investigated the antimicrobial activity of several types of honey, including **Manuka and four regional honeys** (SA13, U4, NJ15, and G2), against members of the ESKAPE group and the environmental bacterium ***Francisella novicida***. *F. novicida* is genetically similar to the highly virulent *F. tularensis* and serves as a model for studying tularemia pathogenesis.

We also examined honey's impact on various ***F. novicida* metabolic mutants** to identify potential bacterial pathways targeted by honey treatment.



Materials and Methods

Honey Samples

Five different honey samples were evaluated for antimicrobial activity: Manuka (commercial medical-grade) and four regional honeys labeled SA13, U4, NJ15, and G2. All honey samples were stored at room temperature in the dark and used without further dilution unless otherwise noted.

Manuka honey

Manuka honey has a well-characterized composition, which is why we selected it for our F. novicida transposon library screening. Our objective is to investigate the effects of MGO (methylglyoxal) and other potentially bioactive, yet unidentified, honey components on specific metabolic pathways involved in Francisella growth.

Bacterial Strains and Culture Conditions

The study included bacterial species from the ESKAPE group as well as *Francisella novicida* U112 (wild-type) and selected metabolic mutants. ESKAPE strains were grown in their respective optimal conditions as recommended by ATCC or strain-specific protocols. *F. novicida* strains were cultured in Tryptic Soy Agar (TSA) at 37°C.

Evaluation of Honey Effects on *F. novicida* Mutants

To investigate potential honey-targeted pathways, *F. novicida* metabolic mutants (e.g., Δ*fslA*, Δ*pilE1*, Δ*hfQ*, etc.) were similarly tested via the zone of inhibition assay. Comparative analysis of zone diameters between wild-type and mutant strains was used to infer differential susceptibility and possible pathway-level interactions.

Results

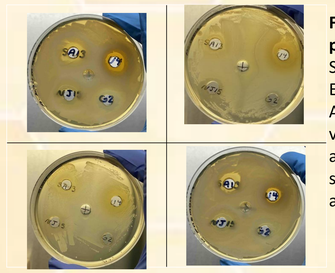


Figure 1. Zone of inhibition produced by regional honeys SA13, U4, NJ15, and G2 against ESKAPE pathogens. All four honeys demonstrated varying degrees of antibacterial activity, with some honeys showing selective efficacy against specific pathogens.

Results

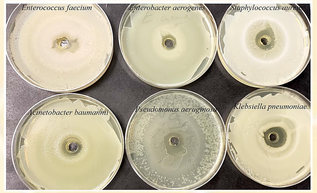
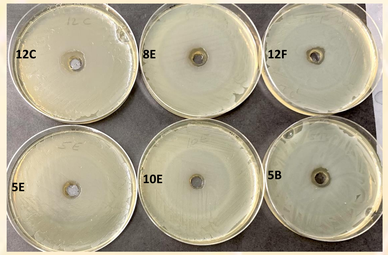


Figure 2. Zone of inhibition for Manuka honey against ESKAPE pathogens. Manuka honey showed strong antimicrobial activity, particularly against Gram-positive strains.

Strain ID	Genotype	Description
KKF253	U112Δ*pilE1-3*::Kan	Defective in pilus biosynthesis
KKF331	U112Δ*fslA*::Kan	Defective in siderophore biosynthesis (*fslA*)
KKF342	U112Δ*hfQ*::Erm	Deletion of pleiotropic regulator HfQ
KKF457	U112ΔFTN_0451–0456::Erm	Defective in c-di-GMP biosynthesis
KKF768	U112Δ*igID* ΔOAgFN::OAgFTT	Attenuated *F. novicida* vaccine strain
U112	*Francisella novicida* U112	Wild-type strain

Figure 4. Comparative zone of inhibition analysis of Manuka honey on *F. novicida* wild-type and various metabolic mutants. Susceptibility differences among mutants suggest that Manuka honey targets specific metabolic pathways. Mutants with reduced zone sizes may indicate possible resistance or altered sensitivity due to pathway disruption.



Well ID	Tn-plate	Locus Tag	Gene/Product - Function / Category
12C	NR-8041	FTN_0606	Hypothetical protein – Unknown
8E	NR-8041	FTN_0936	Hypothetical protein – Unknown
12F	NR-8041	FTN_1298	GTPase (putative enzyme) – Unknown
5E	NR-8041	FTN_0058	β-fructofuranosidase - Carbohydrate metabolism
10E	NR-8041	FTN_1371	Conserved protein of unknown function
5B	NR-8040	FTN_0189	*priA* (primosomal N) - DNA replication, recombination, and repair

Figure 5. *Francisella novicida* transposon mutant strains used to evaluate differential susceptibility to Manuka honey. Each strain harbors a disruption in a specific gene, including those involved in DNA replication (e.g., *priA*), carbohydrate metabolism (e.g., *β-fructofuranosidase*), and several hypothetical or conserved proteins of unknown function. The gene targets and predicted functions are listed alongside their corresponding well and locus identifiers. This mutant panel was used to identify potential genetic pathways contributing to honey susceptibility.

Results

Figure 3. Antibacterial effects of SA13, U4, NJ15, and G2 on *Francisella novicida* U112 wild-type strain. All four honeys produced zones of inhibition, indicating their antimicrobial activity extends to environmental bacterial models. NJ15 and U4 showed relatively higher activity among the tested honeys.

Conclusions

- Manuka and regional honeys showed antibacterial activity against ESKAPE pathogens and *F. novicida*, with Manuka being the most effective.
- *F. novicida* metabolic mutants may in the future reveal differential susceptibility, indicating that honey targets specific bacterial pathways.
- Transposon mutants will be screened to identify uncharacterized genes that may play a role in resistance to components found in honey.

Future Directions

- We aim to screen thousands of *F. novicida* transposon mutants with Manuka honey to uncover genes involved in resistance to its components.
- Further studies will investigate the molecular mechanisms by which honey affects key metabolic and regulatory pathways in pathogenic bacteria.

References

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Acknowledgements

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