Combined effects of acetic acid or commercial vinegars and medical-grade honey on biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

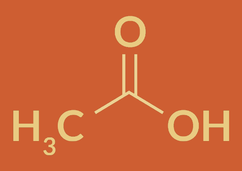
Freya Harrison[1], Anisa Blower[1], Chris de Wolf[1,2], Erin Connelly[1]

[1]School of Life Sciences & [2] Warwick Integrative Synthetic Biology Centre, University of Warwick, Coventry CV4 7AL, United Kingdom

f.harrison@warwick.ac.uk @friendlymicrobe

There is a long history of using vinegar and honey to treat infection. This led to current clinical use of acetic acid and medical-grade honeys to treat wounds infected with bacterial biofilm. There are two gaps in research into these complex natural products. First, other compounds present in vinegar could potentiate or synergise with the action of acetic acid. Second, no research into combined acetic acid + honey therapy has been published.

Clinical use of acetic acid



- Antibacterial at low concentrations; can kill biofilms of Gram-positive and Gram-negative pathogens
- Kills by collapsing the H^+ gradient necessary for ATP synthesis and acidifying the cytoplasm
- 1 in 3 UK burns units use acetic acid-soaked dressings to treat burns infected with *P. aeruginosa*
- Daily dressing of 2.5–3% acetic acid (< many table vinegars) is well-tolerated
- Clinical trials in progress to assess efficacy and optimal dosing

Clinical use of honey

- Manuka (methylglyoxal containing) and non-manuka (peroxide-generating) honeys can kill bacteria
- Methylglyoxal disrupts protein and DNA synthesis and membrane integrity; peroxide produces free radicals that damage many cellular components; all honeys exert some antibacterial effect via osmotic stress and low pH
- Medical-grade honey ointment and honey-impregnated dressings are a common standard of care for wound management
- Indications include treatment for biofilms and infections with high bacterial loads, including diabetic foot ulcers, burns, skin grafts and surgical wounds.

Q1: Is vinegar more antibacterial than pure acetic acid?

Rationale: It is increasingly recognised that many plant extracts and products may owe their antibacterial activity to combinations of compounds

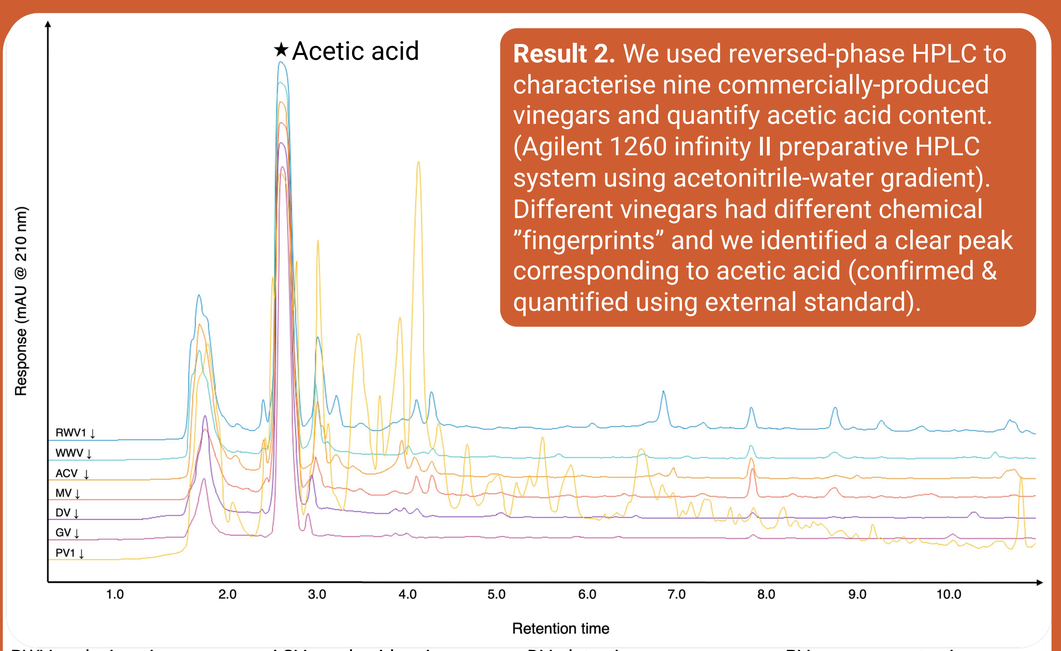
This is why whole honey is still used clinically – the activity cannot be partitioned into a single efficacious component

Q2: What happens when acetic acid or vinegar is combined with honey?

Rationale: in historical medical texts, honey was often mixed with vinegar to make *oxymel*

Some vinegars are more bactericidal than an equivalent dose of acetic acid

Result 1. We performed a systematic review of published studies testing the antibacterial activity of vinegars using MIC or disk diffusion assays. Reporting of quality parameters (no. of replicates, presence of appropriate controls) was generally poor. None of the included studies quantified acetic acid in the vinegars.



Result 2. We used reversed-phase HPLC to characterise nine commercially-produced vinegars and quantify acetic acid content. (Agilent 1260 infinity II preparative HPLC system using acetonitrile-water gradient). Different vinegars had different chemical "fingerprints" and we identified a clear peak corresponding to acetic acid (confirmed & quantified using external standard).

RWV: red wine vinegar
WWV: white wine vinegar
ACV: apple cider vinegar
MV: mead vinegar
DV: date vinegar
GV: grape vinegar
PV: pomegranate vinegar

Result 3. We assayed activity of the vinegars and acetic acid against *S. aureus* and *P. aeruginosa* (example Gram+ and Gram— wound pathogens) using MIC by microdilution in cation-adjusted Muller-Hinton broth (caMHB) and synthetic wound fluid (SWF). Additional bottles of PV and RWV were purchased (different manufacturers), and biofilm eradication assays were conducted for both PVs and RWVs using 24-hr biofilms grown in a collagen-based synthetic wound model. Blue text denotes greater activity than pure acetic acid.

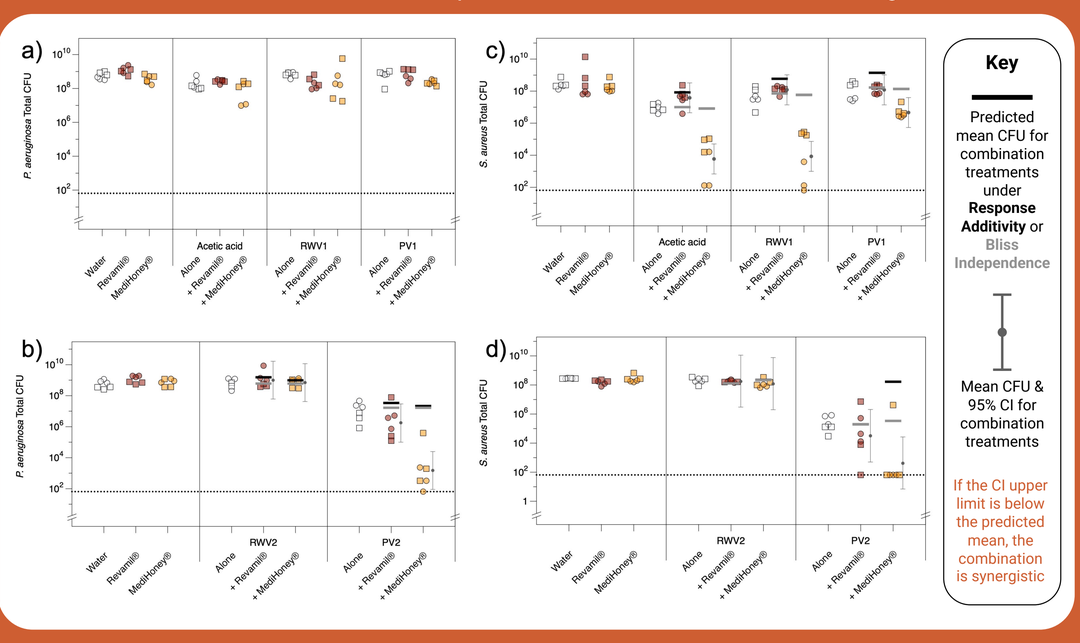
	S. aureus Newman			*P. aeruginosa* PA14		
	MIC in caMHB	MIC in SWF	Biofilm EC_{50}	MIC in caMHB	MIC in SWF	Biofilm EC_{50}
	Units: % acetic acid equivalent			*Units: % acetic acid equivalent*		
Acetic acid	0.38	0.75	0.53	0.38	0.38	0.43
ACV	**0.32**	**0.63**	nd	**0.32**	**0.32**	nd
DV	**0.32**	**0.64**	nd	**0.16**	0.64	nd
GV	0.40	**0.40**	nd	**0.20**	**0.20**	nd
MV	**0.08**	**0.16**	nd	**0.16**	**0.16**	nd
PV1	0.48	**0.48**	0.94	**0.24**	**0.24**	0.96
PV2	nd	nd	**0.28**	nd	nd	**0.01**
RWV1	0.37	**0.37**	0.79	**0.19**	0.37	0.72
RWV2	nd	nd	0.96	nd	nd	0.89
WWV	**0.32**	**0.32**	nd	**0.16**	**0.32**	nd

Additive and synergistic antibiofilm effects of honey + acetic acid and honey + vinegar

Our systematic review found no papers testing synergy of acetic acid / vinegar with honey.

We grew 24-hr biofilms of *S. aureus* and *P. aeruginosa* in a collagen-based synthetic wound model. Biofilms were treated with sub-eradication concentrations of acetic acid, PV, RWV or two medical-grade honey gels: manuka-based MediHoney® or peroxide-generating Revamil®. Additional biofilms were treated with pairwise combinations of acetic acid / PV / RWV plus MediHoney® / Revamil®. In all treatments, acetic acid / vinegar was supplied at a concentration of 0.5% acetic acid or equivalent, and honey gels were supplied at a concentration of 30% wt/vol. After 24h, viable bacteria in the wounds were enumerated.

Two different null models were used to assess the combined effect of the treatments: response additivity and Bliss independence. These make slightly different assumptions about the mechanisms of action and dose-response curves of co-administered drugs.



- **For *P. aeruginosa* treated with acetic acid, PV1 or RWV1 ± honey (a)**, ANOVA revealed no significant interaction between acid treatment and honey treatment, i.e. the effect of acetic acid / vinegar treatment did not depend on the presence of honey.
- **For *P. aeruginosa* treated with PV2 or RWV2 ± honey (b)**, ANOVA revealed a significant interaction between acid treatment and honey treatment. PV2+MediHoney® showed synergistic activity; all other combinations were additive.
- **For *S. aureus* treated with acetic acid, PV1 or RWV1 ± honey (c),** ANOVA revealed a significant interaction between acid treatment and honey treatment. Acetic acid, PV1 or RWV1 showed synergistic activity with MediHoney® but not with Revamil®
- **For *S. aureus* treated with PV2 or RWV2 ± honey (d),** ANOVA revealed a significant interaction between acid treatment and honey treatment. PV2+MediHoney® showed synergistic activity; all other combinations were additive.

- **Some vinegars have antibacterial activity exceeding that predicted by their acetic acid content alone**
- **Pomegranate vinegars are interesting candidates for further study – do they contain molecules with clinical potential?**
- **Acetic acid and vinegars in combination with medical-grade honey products showed additive or synergistic antibiofilm activity**
- **Could the historical combination of vinegar+honey help us develop better advanced wound dressings?**

Key References

Acetic acid clinical potential and use: DOI 10.1371/journal.pone.0136190; https://srmrc.nihr.ac.uk/trials/acetica/
Example good quality study of vinegar antimicrobial activity: DOI 10.3390/molecules27030770
Synergy testing and null models: DOI 10.1016/j.crphar.2022.100110
Synthetic wound model: DOI 10.1111/j.1600-0463.2009.02580.x

Full study available as a preprint: www.biorxiv.org/content/10.1101/2023.04.03.535340