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Antibacterial Screening of Honey Samples From Western Ghats Apiaries Against Wound Pathogens

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ABSTRACT

Honey has been put on wounds for far longer than there have been antibiotics, and laboratory screens of honey against wound pathogens are now routine in many countries, including India. This narrative review asks what such a screen can and cannot tell us, using the published literature on the antibacterial components of honey, on the organisms that colonise and infect wounds, and on the design of susceptibility testing. No new laboratory data are reported, and no data from the apiaries of the Western Ghats are presented. Honey acts through several routes at once: a high sugar content, acidity, hydrogen peroxide generated by enzymatic activity, methylglyoxal in some floral types, the bee-derived peptide defensin-1, and plant phenolics. These routes respond differently to dilution, heat, light and floral origin, so the same jar can look potent in one assay and unremarkable in another. Studies of wound isolates, including methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*, generally report inhibition, and several describe effects on biofilms and cell division, yet the studies differ in honey type, dilution, medium and inoculum, which makes comparison hard. Clinical evidence for honey dressings is more modest than the laboratory literature suggests. The review concludes that a screening result is a useful first filter for regional honeys, provided the honey is characterised, the assay is standardised and the claim is kept to the laboratory, and it lists the information a credible regional screen should report.

Keywords: Honey; Antibacterial activity; Wound pathogens; Western Ghats; Methylglyoxal; Hydrogen peroxide; Susceptibility testing; Narrative review

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Article Class: Narrative Review

1. Introduction

Honey has been laid on wounds for as long as people have recorded how they treated them, and the practice never quite

disappeared, even after antibiotics arrived (1, 2). In the last few decades it has returned to clinical attention, partly because wound infections are increasingly



difficult to treat and partly because laboratory work has shown that honey can inhibit bacteria that are resistant to several drugs (3, 4). Laboratories in many countries now screen local honeys against wound pathogens as a matter of course, and India, with its varied flora and a long tradition of beekeeping, is no exception.

The Western Ghats are a good example of why such screening is tempting. The range is one of the recognised biodiversity hotspots (5), and bees working it forage across a wide mixture of flowering plants through the year. A honey from such an apiary may differ chemically from a honey harvested a few hundred kilometres away, and it is reasonable to ask whether it inhibits the bacteria that infect wounds. The difficulty is that the question is easier to ask than to answer well. A zone on an agar plate looks like a finding, yet what it means depends on the honey, the dilution, the medium and the organism.

This article is a narrative review prompted by the title topic. It draws on published work on the antibacterial components of honey, on wound microbiology and on the design of susceptibility testing. It reports no new laboratory data, and it presents no measurements from any apiary in the Western Ghats or elsewhere; where regional evidence is thin, we say so. The question is what a screening of regional honeys against wound pathogens can legitimately claim, and what it must report to be believed. The aim is a fair account of what a bench result means and what it does not.

2. Literature Review

2.1 How Honey Inhibits Bacteria

Honey is a concentrated sugar solution that bees modify with enzymes of their own, and its antibacterial effect is not a single property. Early surveys found that activity varied widely between honeys and, in

many cases, fell sharply when the honey was heated or diluted (6, 7). The first explanation to hold was enzymatic: a glucose oxidase added by the bee generates hydrogen peroxide when honey is diluted, and this peroxide accounts for much of the activity in many samples (8). The osmotic effect of the sugar, the acidity of the honey and the plant phenolics it carries add to this, in proportions that depend on the honey (Table 1) (9, 10).

Peroxide is a fragile mechanism. Catalase from plant pollen or other sources can remove it, and heat and light degrade the enzyme that makes it (11). The rate at which peroxide accumulates also depends on how much the honey has been diluted, which has direct consequences for any wound application (12). Later work questioned whether peroxide alone could explain the activity of some honeys, and argued that its role had been simplified in earlier accounts (13).

Table 1. Antibacterial components of honey and how they behave.

Component	Source	Behaviour
Sugar content	Nectar, concentrated in the hive	Osmotic effect; weakens as honey is diluted (7, 10)
Acidity	Organic acids, gluconic acid	Contributes; buffered in tissue (7, 9)
Hydrogen peroxide	Bee glucose oxidase	Made on dilution; lost with heat, light, catalase (8, 11, 13)
Methylglyoxal	Floral precursor (manuka)	Heat-stable; non-peroxide activity (14)
Defensin-1	Bee secretion	Peptide; found in several honeys (15, 16)
Phenolics	Plant material	Vary with floral source (9)

Note: Qualitative summary of the cited reviews. Which components matter most differs between honeys.



Some honeys retain activity when peroxide is neutralised. In manuka honey from New Zealand, methylglyoxal, formed from a precursor in the nectar, was identified as the dominant antibacterial constituent (14). Other work on medicinal honeys found that the bee-derived peptide defensin-1 contributes to the effect, and that different honeys kill bacteria by different routes (15, 16). In practice this means that two honeys with similar numerical activity can behave differently once diluted, stored or applied to tissue, a point that matters for any comparison between floral types.

2.2 Wound Pathogens

Wounds are colonised by a shifting community, and the organisms that matter depend on the wound. *Staphylococcus aureus* is a leading cause of infection after surgery and trauma, and the methicillin-resistant lineages are a particular concern (17, 18). *Pseudomonas aeruginosa* is notorious in burns and chronic wounds, where it is hard to clear and often forms biofilms (19, 20). Streptococci, enterococci and enteric bacteria such as *Escherichia coli* complete a list that a screen of honey against wound pathogens would normally contain (Table 2).

Biofilms deserve separate mention. Bacteria in biofilms are embedded in a matrix and tolerate drugs that kill the same organisms grown in suspension (21), and chronic wounds frequently contain them (22). A susceptibility result for planktonic cells therefore says little about whether a honey would work against organisms in the form in which they persist in a wound, and a swab of the surface can miss organisms deeper in the tissue. A clinician reading such a report needs the answer to a plain question: would this honey do the same thing in a wound?

Table 2. Wound pathogens and what the honey literature says about them.

Organism	Wound relevance	Honey literature
<i>Staphylococcus aureus</i>	Common in surgical and chronic wounds (17, 18)	Inhibited, including MRSA (23, 24)
<i>Pseudomonas aeruginosa</i>	Burns; biofilm former (19, 20)	Inhibited in burn isolates (25, 26)
<i>Streptococcus pyogenes</i>	Skin and soft tissue infection	Biofilm effects reported (27)
Enterococci, coagulase-negative staphylococci	Gram-positive cocci in wounds	Sensitive in the cited survey (28)
<i>Escherichia coli</i>	Enteric, contaminates wounds	Inhibited in several screens (29, 30)
Mixed biofilms	Chronic wounds (21, 22)	Evidence is early (31)

Note: Qualitative reading of the cited studies; no inhibition values are quoted because they depend on assay conditions.

2.3 Reports of Activity Against Wound Isolates

Several groups tested honey against organisms taken from wounds rather than against reference strains alone. One survey of *Staphylococcus aureus* from infected wounds found inhibition by honey across the isolates tested (23), and a related study of other Gram-positive cocci found them sensitive as well (28). Isolates of *Pseudomonas aeruginosa* from burns were also inhibited (25). Comparisons between honeys from different regions, including Malaysian tualang honey, Chilean ulmo honey and honeys from other countries, reported activity against wound and enteric bacteria in several cases (26, 29, 30).

Work on processed and raw honeys has tried to compare commercial products with unprocessed ones, with the sensible



concern that heating and filtration may remove activity (32). A study of honey from Australian flora found activity in many floral types and a wide spread between them (33). The recurring pattern is that inhibition is common and potency is variable, which is exactly what the mixture of mechanisms in Section 2.1 would predict. This is why one honey can look excellent in one report and ordinary in the next.

2.4 Cells, Biofilms and Resistance

Beyond simple inhibition, laboratory studies have asked what honey does to the cell. Manuka honey was reported to interfere with cell division in methicillin-resistant *Staphylococcus aureus* (24) and to affect intracellular processes in the same species (34). It reduced the development of *Streptococcus pyogenes* biofilms and the expression of two surface proteins (27), and manuka-type honeys were able to eradicate *Staphylococcus aureus* biofilms in vitro (31). These are mechanistic findings in controlled conditions, and they should be read as such.

A frequent claim is that bacteria do not develop resistance to honey. The evidence behind this is more careful than the slogan: laboratory attempts to select resistant mutants of wound pathogens were unsuccessful (35), and a study of repeated exposure found no resistance to medical-grade manuka honey (36). That is reassuring, but it is a finding about particular honeys and particular strains, not a guarantee that applies to every honey, and the multiple mechanisms of honey are the likelier reason for the observation than any property of the bacteria.

2.5 Clinical Evidence

Laboratory activity does not guarantee clinical benefit. Reviews of honey as a wound dressing have described

encouraging results in some wound types and acknowledged that the trials are often small and heterogeneous (37, 38). The Cochrane review of honey as a topical treatment found that evidence was of low quality for most comparisons, with the clearest signal in superficial and partial-thickness burns, and little to show that honey consistently outperformed conventional dressings in other wounds (39). A bench screen of regional honeys therefore sits one long step away from a clinical claim.

3. Materials and Methods

3.1 Scope and Question

The review asks a single question in two parts: what can a laboratory screen of honey samples from a defined region against wound pathogens legitimately claim, and what must it report so that others can use the result? The question was broken into the issues that a screen has to settle: which honeys were tested and how they were handled, which organisms were used and where they came from, how inhibition was defined, and how the results relate to the conditions of a wound.

3.2 Literature Assembly

The review draws on peer-reviewed work in four areas: the chemistry of antibacterial activity in honey, wound microbiology, in vitro studies of honey against wound isolates and biofilms, and clinical reviews of honey dressings. Sources were chosen for relevance, citation in the field and clarity of methods, and work published after 2015 is not considered. Studies of honeys from other countries are used to illustrate mechanisms and methods, and no result from them is assumed to hold for the honeys of the Western Ghats.

We did not run a formal systematic search and do not claim one. This is a narrative synthesis, and the choice of sources reflects the authors' judgement. The



review is therefore a better guide to what a good screen should contain than to the exact size of any reported effect, and it avoids quoting inhibition values from individual studies that could not be checked against the primary reports.

3.3 Appraisal Framework

To judge how far a report can be trusted, we used four questions. Was the honey characterised, in terms of floral origin, handling and storage (11, 40)? Was the assay standardised, with a stated inoculum, medium and endpoint (41, 42)? Was the peroxide contribution separated from the rest, for instance by treating with catalase (16)? And were the organisms relevant to wounds, including isolates and, where claimed, biofilms (23, 31)? Each question was applied to what a report actually states, not to what it might be assumed to have done. Table 3 lists the variables that these questions cover. None of the questions is a quality score. None of these steps is difficult, but each is easy to skip.

Table 3. Variables that decide whether two honey screens can be compared.

Variable	Why it matters	What to report
Floral origin	Changes the components present (33, 40)	Source, region, season
Handling and storage	Heat and light alter activity (11)	Time, temperature, light
Dilution	Activates peroxide, weakens osmosis (12)	Percent used, diluent
Peroxide control	Separates peroxide from other effects (16)	Catalase treatment
Inoculum and medium	Alter apparent potency (41)	Density, broth or agar
Endpoint	Growth, kill or biofilm (31)	Definition of inhibition

Note: The variables are those discussed in the cited methodological literature.

4. Results

4.1 What a Regional Honey Brings

The literature describes honey as a variable natural product rather than a standard reagent. Its sugars and acids are broadly similar across honeys, but the minor components that drive much of the inhibition, peroxide, methylglyoxal, defensin-1 and phenolics, differ with the floral source and with what the bees add (10, 33). For the Western Ghats this has a specific implication: a mixed-flora honey from a hotspot is not interchangeable with a unifloral honey from elsewhere, and reports on the one cannot simply be applied to the other (5, 40).

A regional screen could in principle find activity from any of the routes shown in Figure 1, and a positive result does not say which. Without a peroxide control and some account of the floral origin, the best that can be said is that a sample inhibited a strain under stated conditions. Even that statement is useful, provided the conditions are stated. Read this way, a weak result in a single assay is not proof that a honey is inactive.

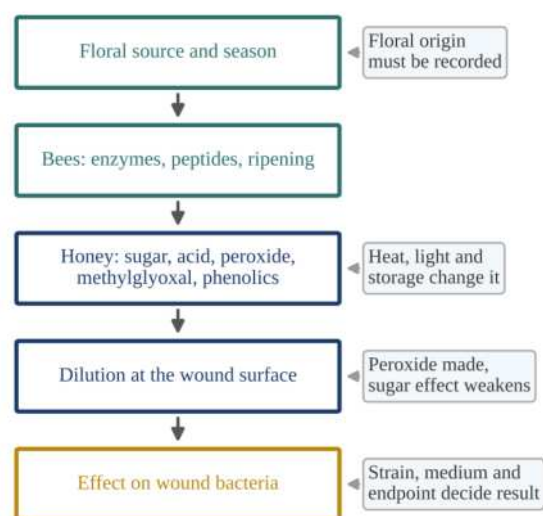


Figure 1. From floral source to a wound surface, with the points where activity can change (schematic, not data).



4.2 Where Results Diverge

Different routes behave differently under handling, as [Figure 2](#) summarises. Dilution weakens the osmotic effect, produces peroxide and leaves methylglyoxal largely untouched, while heat and light can destroy the enzymatic route ([11](#), [12](#)). This is the reason a honey that was stored carelessly may appear inactive in an assay that would have shown activity on the day it was harvested. A honey kept warm in a bright room behaves differently from one kept cool and dark.

Assay design adds a second layer. Agar diffusion and broth dilution answer different questions, with diffusion depending on how well a component moves through the medium and broth methods giving a concentration at which growth stops ([41](#), [42](#)). honeys that look alike by one method may be separated by the other, and results expressed as a zone diameter cannot be set beside results expressed as a percentage concentration. The differences look small one at a time, yet they decide whether two laboratories can ever agree.

4.3 Dilution and the Wound

Percentage concentrations in a laboratory are not what a wound sees. Wound exudate dilutes honey, and as it does the osmotic effect fades while peroxide production rises and then falls as the honey thins out ([12](#)). A screen that reports activity only for the undiluted honey or only for a strongly diluted one has told us about the assay, not about the dressing. A result obtained at a dilution no wound would ever see is not one a patient would experience. A fair screen reports a range of dilutions, shows the effect of removing peroxide, and says how the dilutions map onto any application the authors have in mind ([13](#), [16](#)). Contact time matters too, since a dressing stays on for hours.

	Dilution	Heat, light	Floral source
Sugar (osmotic)	Weakens	Little	Slight
Acidity	Buffered	Little	Some
Peroxide	Generated	Easily lost	Some
Methylglyoxal	Retained	Stable	Strong
Defensin-1	Retained	Uncertain	Unclear
Phenolics	Retained	Some loss	Strong

Figure 2. Qualitative matrix of how each antibacterial route responds to common handling (schematic, not data).

4.4 Quality of the Evidence

Reports of honey screening share a set of limitations ([Table 3](#)). The honey is often described only by region or by the beekeeper's name, so that another laboratory could not obtain the same product. Strains are chosen for convenience, sometimes reference strains alone, so that the link to real wounds is inferred rather than shown ([23](#), [29](#)). And replication is frequently thin, with a handful of plates per honey and no indication of variation between jars or harvests. The point is not that a screen is poor, only that its result travels less far than its abstract suggests.

There is also a mismatch of ambition. The clinical literature is cautious ([39](#)), yet the laboratory literature is frequently written as if inhibition on a plate were a step towards a dressing. The genuine strength of the field is the convergence of mechanisms, traced in [Figure 3](#), and the consistent finding of activity against wound isolates, including resistant ones ([3](#), [35](#)). The weakness is that these findings come mostly from a small number of well-characterised honeys, and the Western Ghats honeys are not among them.



Put plainly, the literature supports only a cautious statement. Honey inhibits many wound pathogens in vitro, its activity depends strongly on floral origin and handling, and the translation to clinical benefit is not established for most wound types. Whether a given regional honey will do the same is an empirical question that no review can settle. That is a modest claim, but it is the one the literature currently supports.

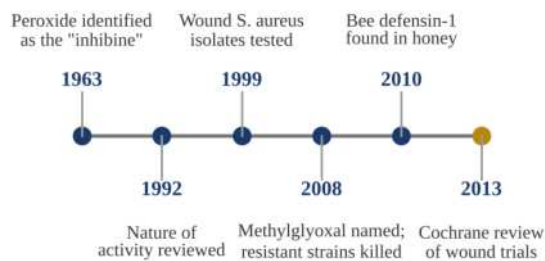


Figure 3. Timeline of conceptual milestones cited in this review (schematic; spacing is not to scale).

4.5 Reading a Claim of Activity

A reader meeting a claim that a regional honey is antibacterial can ask a short sequence of questions. How was the honey obtained, handled and stored, and is its floral origin stated (40)? How were the bacteria grown and the endpoint defined (42)? Was a control for peroxide run, and was a reference honey of known activity included (14, 16)? Were the strains from wounds, and were the results reported with replicates? Answers to these questions, more than the size of a zone, determine how much weight a claim can carry. It is a habit worth keeping even when the answer is expected.

5. Discussion

5.1 Interpretation and Practical Reading

The main finding of this review is a conditional one. Honey is likely to inhibit wound pathogens in vitro, but the extent

depends on the route that dominates in a particular honey, and that in turn depends on floral origin and handling (6, 10). For a hotspot region with mixed flora, this means that a screen is best treated as a first filter, one that identifies samples worth characterising further, and not as a final judgement. Nothing in this reading argues against testing regional honeys; it argues for describing them properly when they are tested.

The second conditional concerns the clinical step. The laboratory evidence, however consistent, does not by itself justify putting an unprocessed regional honey on wounds. Medical-grade honeys are standardised, sterilised and tested, and the trials that support dressings were done with such products (37, 39). Raw honey may carry bacterial spores and other contaminants, and a screen that finds activity should be followed by questions about safety and sterility, not by a recommendation for use. We make no individual advice here and note only that decisions on wound care belong with clinicians.

5.2 Limitations

This is a narrative review and shares the limitations of that form. The selection of sources reflects judgement, no formal search or quality scoring was done, and the review quotes no numerical results from individual studies because these could not be checked against the primary reports for this article. A reader who needs an effect size should consult the original studies directly.

The review also draws mostly on honeys from other regions. Studies of manuka and other well-known honeys describe those honeys, and the transfer to mixed-flora honey from the Western Ghats is a hypothesis, not a finding. We have used them to frame what a regional screen should report, and the conclusions should



be read in that light. Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

5.3 Directions for Future Work

Three needs stand out. First, regional screens should characterise each honey, with floral origin recorded and a peroxide control included, and should report results at several dilutions using a standard method (41, 42). Second, wound isolates and biofilms should be included where wound relevance is claimed (22, 31). Third, a systematic review of in vitro honey screens, with transparent search and appraisal, would show how much of the literature can be combined at all. These points apply with equal force to any natural product screened against bacteria, not only to honey.

6. Conclusion

Honey is a plausible and well-documented inhibitor of wound pathogens in the laboratory, and a screen of honeys from the Western Ghats apiaries against such organisms is a reasonable first step. What the literature does not allow is the leap from a clear zone on a plate to a claim about wounds. A credible regional screen describes its honey, standardises its assay, controls for peroxide, tests clinically relevant organisms and keeps its claims inside the laboratory.

For the investigator, the message is practical rather than discouraging. Regional honeys are worth testing, provided the honey is characterised, the method is stated, the dilutions are realistic and the limits of the result are written down. For the clinician and the reader, the sensible position is interest without haste: promising in vitro activity is a reason for further study and not, on its own, a reason for treatment.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

R.A. conceived the review and wrote the sections on honey chemistry and screening methods. M.V. wrote the sections on wound microbiology and biofilms. P.M. wrote the sections on clinical evidence and appraisal. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human participants or animals were used and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

13. References

1. Molan, P. C. (1999). The role of honey in the management of wounds. *Journal of Wound Care*, 8(8), 415–418. DOI: [10.12968/jowc.1999.8.8.25904](https://doi.org/10.12968/jowc.1999.8.8.25904)
2. Bogdanov, S., Jurendic, T., Sieber, R., & Gallmann, P. (2008). Honey for nutrition and health: A review. *Journal of the American College of Nutrition*, 27(6), 677–689. DOI: [10.1080/07315724.2008.10719745](https://doi.org/10.1080/07315724.2008.10719745)
3. Kwakman, P. H. S., te Velde, A. A., de Boer, L., Vandenbroucke-Grauls, C. M. J. E., & Zaat, S. A. J. (2008). Medical-grade honey kills antibiotic-resistant bacteria in vitro and eradicates skin colonization. *Clinical Infectious Diseases*, 46(11), 1677–1682. DOI: [10.1086/587901](https://doi.org/10.1086/587901)
4. Maddocks, S. E., & Jenkins, R. E. (2013). Honey: A sweet solution to the growing problem of antimicrobial resistance? *Future Microbiology*, 8(11), 1419–1429. DOI: [10.2217/fmb.13.105](https://doi.org/10.2217/fmb.13.105)



5. Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403(6772), 853–858. DOI: [10.1038/35002501](https://doi.org/10.1038/35002501)
6. Allen, K. L., Molan, P. C., & Reid, G. M. (1991). A survey of the antibacterial activity of some New Zealand honeys. *Journal of Pharmacy and Pharmacology*, 43(12), 817–822. DOI: [10.1111/j.2042-7158.1991.tb03186.x](https://doi.org/10.1111/j.2042-7158.1991.tb03186.x)
7. Molan, P. C. (1992). The antibacterial activity of honey: 1. The nature of the antibacterial activity. *Bee World*, 73(1), 5–28. DOI: [10.1080/0005772X.1992.11099109](https://doi.org/10.1080/0005772X.1992.11099109)
8. White, J. W., Subers, M. H., & Schepartz, A. I. (1963). The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochimica et Biophysica Acta*, 73(1), 57–70. DOI: [10.1016/0006-3002\(63\)90363-8](https://doi.org/10.1016/0006-3002(63)90363-8)
9. Mandal, M. D., & Mandal, S. (2011). Honey: Its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, 1(2), 154–160. DOI: [10.1016/S2221-1691\(11\)60016-6](https://doi.org/10.1016/S2221-1691(11)60016-6)
10. Kwakman, P. H. S., & Zaat, S. A. J. (2012). Antibacterial components of honey. *IUBMB Life*, 64(1), 48–55. DOI: [10.1002/iub.578](https://doi.org/10.1002/iub.578)
11. Weston, R. J. (2000). The contribution of catalase and other natural products to the antibacterial activity of honey: A review. *Food Chemistry*, 71(2), 235–239. DOI: [10.1016/S0308-8146\(00\)00162-X](https://doi.org/10.1016/S0308-8146(00)00162-X)
12. Bang, L. M., Bunting, C., & Molan, P. (2003). The effect of dilution on the rate of hydrogen peroxide production in honey and its implications for wound healing. *Journal of Alternative and Complementary Medicine*, 9(2), 267–273. DOI: [10.1089/10755530360623383](https://doi.org/10.1089/10755530360623383)
13. Brudzynski, K., Abubaker, K., St-Martin, L., & Castle, A. (2011). Re-examining the role of hydrogen peroxide in bacteriostatic and bactericidal activities of honey. *Frontiers in Microbiology*, 2, 213. DOI: [10.3389/fmicb.2011.00213](https://doi.org/10.3389/fmicb.2011.00213)
14. Mavric, E., Wittmann, S., Barth, G., & Henle, T. (2008). Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Molecular Nutrition & Food Research*, 52(4), 483–489. DOI: [10.1002/mnfr.200700282](https://doi.org/10.1002/mnfr.200700282)
15. Kwakman, P. H. S., te Velde, A. A., de Boer, L., Speijer, D., Vandenbroucke-Grauls, C. M. J. E., & Zaat, S. A. J. (2010). How honey kills bacteria. *The FASEB Journal*, 24(7), 2576–2582. DOI: [10.1096/fj.09-150789](https://doi.org/10.1096/fj.09-150789)
16. Kwakman, P. H. S., te Velde, A. A., de Boer, L., Vandenbroucke-Grauls, C. M. J. E., & Zaat, S. A. J. (2011). Two major medicinal honeys have different mechanisms of bactericidal activity. *PLoS ONE*, 6(3), e17709. DOI: [10.1371/journal.pone.0017709](https://doi.org/10.1371/journal.pone.0017709)
17. Lowy, F. D. (1998). *Staphylococcus aureus* infections. *New England Journal of Medicine*, 339(8), 520–532. DOI: [10.1056/NEJM199808203390806](https://doi.org/10.1056/NEJM199808203390806)
18. Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews*, 14(2), 244–269. DOI: [10.1128/CMR.14.2.244-269.2001](https://doi.org/10.1128/CMR.14.2.244-269.2001)
19. Church, D., Elsayed, S., Reid, O., Winston, B., & Lindsay, R. (2006). Burn wound infections. *Clinical Microbiology Reviews*, 19(2), 403–434. DOI: [10.1128/CMR.19.2.403-434.2006](https://doi.org/10.1128/CMR.19.2.403-434.2006)
20. Lyczak, J. B., Cannon, C. L., & Pier, G. B. (2000). Establishment of *Pseudomonas aeruginosa* infection: Lessons from a versatile opportunist. *Microbes and Infection*, 2(9), 1051–1060. DOI: [10.1016/S1286-4579\(00\)01259-4](https://doi.org/10.1016/S1286-4579(00)01259-4)
21. Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: A common cause of persistent infections. *Science*, 284(5418), 1318–1322. DOI: [10.1126/science.284.5418.1318](https://doi.org/10.1126/science.284.5418.1318)
22. James, G. A., Swogger, E., Wolcott, R., Pulcini, E., deLancey, Secor, P., Sestrich, J., Costerton, J. W., & Stewart, P. S. (2008). Biofilms in chronic wounds. *Wound Repair and Regeneration*, 16(1), 37–44. DOI: [10.1111/j.1524-475X.2007.00321.x](https://doi.org/10.1111/j.1524-475X.2007.00321.x)
23. Cooper, R. A., Molan, P. C., & Harding, K. G. (1999). Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *Journal of the Royal Society of Medicine*, 92(6), 283–285. DOI: [10.1177/014107689909200604](https://doi.org/10.1177/014107689909200604)
24. Jenkins, R., Burton, N., & Cooper, R. (2011). Manuka honey inhibits cell division in methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, 66(11), 2536–2542. DOI: [10.1093/jac/dkr340](https://doi.org/10.1093/jac/dkr340)
25. Cooper, R. A., Halas, E., & Molan, P. C. (2002). The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. *Journal of Burn Care & Rehabilitation*, 23(6), 366–370. DOI: [10.1097/00004630-200211000-00002](https://doi.org/10.1097/00004630-200211000-00002)
26. Sherlock, O., Dolan, A., Athman, R., Power, A., Gethin, G., Cowman, S., & Humphreys, H. (2010). Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complementary and Alternative Medicine*, 10, 47. DOI: [10.1186/1475-2875-10-47](https://doi.org/10.1186/1475-2875-10-47)



- [10.1186/1472-6882-10-47](https://doi.org/10.1186/1472-6882-10-47)
27. Maddocks, S. E., Lopez, M. S., Rowlands, R. S., & Cooper, R. A. (2012). Manuka honey inhibits the development of *Streptococcus pyogenes* biofilms and causes reduced expression of two fibronectin binding proteins. *Microbiology*, 158(3), 781–790. DOI: [10.1099/mic.0.053959-0](https://doi.org/10.1099/mic.0.053959-0)
 28. Cooper, R. A., Molan, P. C., & Harding, K. G. (2002). The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *Journal of Applied Microbiology*, 93(5), 857–863. DOI: [10.1046/j.1365-2672.2002.01770.x](https://doi.org/10.1046/j.1365-2672.2002.01770.x)
 29. Tan, H. T., Rahman, R. A., Gan, S. H., Halim, A. S., Hassan, S. A., Sulaiman, S. A., & Kirpal-Kaur, B. (2009). The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC Complementary and Alternative Medicine*, 9, 34. DOI: [10.1186/1472-6882-9-34](https://doi.org/10.1186/1472-6882-9-34)
 30. Basualdo, C., Sgroy, V., Finola, M. S., & Marioli, J. M. (2007). Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Veterinary Microbiology*, 124(3–4), 375–381. DOI: [10.1016/j.vetmic.2007.04.039](https://doi.org/10.1016/j.vetmic.2007.04.039)
 31. Lu, J., Turnbull, L., Burke, C. M., Liu, M., Carter, D. A., Schlothauer, R. C., Whitchurch, C. B., & Harry, E. J. (2014). Manuka-type honeys can eradicate biofilms produced by *Staphylococcus aureus* strains with different biofilm-forming abilities. *PeerJ*, 2, e326. DOI: [10.7717/peerj.326](https://doi.org/10.7717/peerj.326)
 32. Mohapatra, D. P., Thakur, V., & Brar, S. K. (2011). Antibacterial efficacy of raw and processed honey. *Biotechnology Research International*, 2011, 917505. DOI: [10.4061/2011/917505](https://doi.org/10.4061/2011/917505)
 33. Irish, J., Blair, S., & Carter, D. A. (2011). The antibacterial activity of honey derived from Australian flora. *PLoS ONE*, 6(3), e18229. DOI: [10.1371/journal.pone.0018229](https://doi.org/10.1371/journal.pone.0018229)
 34. Henriques, A. F., Jenkins, R. E., Burton, N. F., & Cooper, R. A. (2010). The intracellular effects of manuka honey on *Staphylococcus aureus*. *European Journal of Clinical Microbiology & Infectious Diseases*, 29(1), 45–50. DOI: [10.1007/s10096-009-0817-2](https://doi.org/10.1007/s10096-009-0817-2)
 35. Blair, S. E., Cokcetin, N. N., Harry, E. J., & Carter, D. A. (2009). The unusual antibacterial activity of medical-grade *Leptospermum* honey: Antibacterial spectrum, resistance and transcriptome analysis. *European Journal of Clinical Microbiology & Infectious Diseases*, 28(10), 1199–1208. DOI: [10.1007/s10096-009-0763-z](https://doi.org/10.1007/s10096-009-0763-z)
 36. Cooper, R. A., Jenkins, L., Henriques, A. F. M., Duggan, R. S., & Burton, N. F. (2010). Absence of bacterial resistance to medical-grade manuka honey. *European Journal of Clinical Microbiology & Infectious Diseases*, 29(10), 1237–1241. DOI: [10.1007/s10096-010-0992-1](https://doi.org/10.1007/s10096-010-0992-1)
 37. Molan, P. C. (2006). The evidence supporting the use of honey as a wound dressing. *The International Journal of Lower Extremity Wounds*, 5(1), 40–54. DOI: [10.1177/1534734605286014](https://doi.org/10.1177/1534734605286014)
 38. Simon, A., Traynor, K., Santos, K., Blaser, G., Bode, U., & Molan, P. (2009). Medical honey for wound care: Still the “latest resort”? *Evidence-Based Complementary and Alternative Medicine*, 6(2), 165–173. DOI: [10.1093/ecam/nem175](https://doi.org/10.1093/ecam/nem175)
 39. Jull, A. B., Walker, N., & Deshpande, S. (2013). Honey as a topical treatment for wounds. *Cochrane Database of Systematic Reviews*, (2), CD005083. DOI: [10.1002/14651858.CD005083.pub3](https://doi.org/10.1002/14651858.CD005083.pub3)
 40. Persano Oddo, L., & Piro, R. (2004). Main European unifloral honeys: Descriptive sheets. *Apidologie*, 35(Suppl. 1), S38–S81. DOI: [10.1051/apido:2004049](https://doi.org/10.1051/apido:2004049)
 41. Jorgensen, J. H., & Ferraro, M. J. (2009). Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clinical Infectious Diseases*, 49(11), 1749–1755. DOI: [10.1086/647952](https://doi.org/10.1086/647952)
 42. Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3(2), 163–175. DOI: [10.1038/nprot.2007.521](https://doi.org/10.1038/nprot.2007.521)