

Honey as an Antimicrobial Agent Against *Pseudomonas Aeruginosa* Isolated from Infected Wounds

Vishnu Prasad Shenoy, Mamatha Ballal¹, PG Shivananda², Indira Bairy³

Department of Microbiology, Kasturba Medical College, Manipal University, Manipal, ¹Department of Clinical Microbiology and Immunology, KMC International Center, Manipal, ²Chair Microbiology and Incharge Virology Laboratory Manipal University, ³Department of Microbiology, Melaka Manipal Medical College, Manipal Campus Manipal University, Manipal, India

ABSTRACT

Background: As natural products garner attention in the medical field due to emergence of antibiotic resistant strains of bacteria, honey is valued for its antibacterial activity. **Objective:** Fifty strains of *Pseudomonas aeruginosa* isolated from infected wounds were evaluated for their antibacterial action using honey in comparison with different antibiotics and Dettol. **Methodology and Results:** All the strains were found to be sensitive to honey at a minimum inhibitory concentration of 20% in comparison with Dettol at 10% using agar dilution method. In the second step, the time kill assay was performed on five isolates of *P. aeruginosa* to demonstrate the bactericidal activity of honey at different dilutions of honey ranging from 20% to 100% at regular time intervals. All the isolates of *P. aeruginosa* tested were killed in 12-24 h depending on the dilutions of the honey tested. Thus, honey could prevent the growth of *P. aeruginosa* even if it was diluted by deionized water by fivefolds *in vitro*. Honey had almost uniform bactericidal activity against *P. aeruginosa* irrespective of their susceptibility to different classes of antibiotics. **Conclusion:** Honey which is a natural, non-toxic, and an inexpensive product has activity against the *P. aeruginosa* isolated from infected wounds may make it an alternative topical choice in the treatment of wound infections.

Key words: Antibacterial activity, Honey, *P. aeruginosa*

INTRODUCTION

Honey whose medicinal uses date from ancient times has been lately rediscovered as therapy for wounds. The antimicrobial effect of honey has been reported by a number of workers it is commonly used as a base for ointments and has very successfully been applied in surgical dressings for open wounds and burns to avoid septic infections.^[1] The current prevalence of antibiotic-resistant microbial species has led to a re-evaluation of the therapeutic use of ancient remedies, including honey.^[2] Strong solution of honey or sugar and sugar pastes inhibits microbial growth due to high osmolarity but when used as dressings this action ceases.^[3] But such wounds are rapidly rendered sterile by honey because of its additional antimicrobial activity.^[4,5] It has been traditionally used in treatment of burn wound infection in rural India and the study has shown that it is superior to silver sulfadiazine.^[6]

Indiscriminate use of antibiotics has led to the emergence of drug-resistant strains which have a significant impact on patient's morbidity and mortality.^[7] To date, there are many publications on studies performed both *in vitro* and *in vivo* on the therapeutic properties of manuka honey, and have confirmed its activity against a wide range of medically important bacteria.^[8] Honey is produced from many floral sources and its antimicrobial activity varies markedly with its origin and processing.^[9-11] This variation can be due to difference in the enzymatic action and in the presence of additional antibacterial components derived from the floral source.^[12] As the potential role for honey as a topical agent to manage surgical site or wound infections is increasingly acknowledged^[13] other honeys need to be assessed and evaluated.

The present study was to evaluate the antimicrobial activity of honey native to India at various concentrations against *Pseudomonas aeruginosa* causing wound infection and its comparison with antibiotics and Dettol.

MATERIALS AND METHODS

A total of 50 strains of *P. aeruginosa* isolated from different

Address for correspondence:

Dr. Mamatha Ballal, E-mail: mamatha_98@yahoo.com

Access this article online

Quick Response Code:



Website:
www.jgid.org

DOI:
10.4103/0974-777X.96770

types of wound infection including burns wound were used in the study. The isolation and identification of *P. aeruginosa* was done using standard methods.^[14] The antibiotic-sensitivity patterns of the isolates were studied by Kirby Bauer's disc diffusion method using commercially available antibiotic discs (Hi-Media labs, Mumbai India) gentamicin (10 µg), amikacin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), netillin (30 µg), cefotaxime (30 µg), piperacillin (100 µg), and imipenem (10 µg) as per the clinical and laboratory standards institute (CLSI) standards.

Antibacterial effect of honey

Agmark grade honey (Dabur India, Capital overseas), a polyfloral honey with yellowish brown color, was used in the study. It had 100% purity and each 100 g of honey contains 80 g natural sugars, sodium 17 mg, potassium 138 mg, calcium 13 mg, iron 1.5 mg, and phosphorus 5 mg; sterilized by ultrafiltration and have the floral source from Himalayas, Nilgiris, and Sunderbans of India.

The minimum active dilution of honey against *P. aeruginosa* isolates was determined by agar dilution methods.^[3] Double strength nutrient agar was used for the study, measured out into 10 ml aliquots and autoclaved. To prepare the plates it was melted and maintained at a temperature of 50 °C water-bath until poured. Solutions of the honey samples (at a concentration of 50% v/v) were prepared in sterile de-ionized water immediately prior to performing an assay. Appropriate concentrations of the stock and deionized water (total 10 ml) were dispensed aseptically into 10 ml sterile double strength nutrient agar before pouring into Petri dishes to produce a dilution series (5-25%) from concentrations required in a volume of 20 ml. The various agar honey mixtures were then poured into triplicate Petri dishes. Antibacterial action of honey was compared with Dettol, (Reckitt Benckiser India Ltd, Capital Overseas) at a concentration similar to honey which contains an aromatic chemical compound chloroxylenol (C₈H₉ClO) (4.8% of the total Dettol mixture), which is the key ingredient exhibiting unique antiseptic property. Subcultures of the *P. aeruginosa* isolates were grown overnight in 10 ml peptone water. The turbidity was adjusted to 0.5 Mc Farland and 10 µl of culture was spot inoculated on to the surface of the medium. The plates were incubated at 37°C for 24 h before visual assessment of the growth. The tests were performed in triplicates. Control plates of plain nutrient agar were included in each susceptibility assay to confirm the viability and density of the cultures. *P. aeruginosa* american type culture collection (ATCC) strain 27853 was inoculated on each plate for comparison.

The bactericidal effect of honey was studied by time kill assay.^[15] Honey was used undiluted and at different dilutions. With each experiment saline control of the organism was also used. Two milliliters each of undiluted and different dilutions of the honey were taken in sterile test tubes and inoculated with 20 µl of broth culture of the test bacterium in an initial concentration of approximately 5×10⁵ CFU/ml. All the tubes were incubated at 37°C and the viable count of bacteria in each tube was determined at an interval of 4 h up to 24 h by the surface plate method.^[16] Viable count of bacteria in both test and control tubes at each time interval was noted by performing plate culture on nutrient agar without honey.

RESULTS

A total of 50 strains of *P. aeruginosa* isolated from infected wounds were studied over a period of 2 years. The antibiotic susceptibility patterns of the *P. aeruginosa* isolates are shown in Figure 1, where the y axis indicates the percentage of the isolates susceptible to various antibiotics plotted on the X axis. Multidrug resistance was a common feature among most of these isolates 41 (82%) and the remaining 9 (18%) isolates were pan sensitive. Among the antibiotics tested against *P. aeruginosa* isolates in this study imipenem 50 (100%) being highly effective followed by piperacillin 39 (78%), amikacin 38 (76%) netillin 32 (64%), ceftazidime 28 (56%), gentamicin 23 (46%), cefotaxime 23 (46%), and ciprofloxacin 20 (40%), respectively.

The effects of honey on all the isolates of *P. aeruginosa* were studied by the determination of minimum inhibitory concentration (MIC), indicating the highest dilution of honey in the culture medium which inhibits the growth of *P. aeruginosa* isolates. The MIC of all the 50 strains (100%) to honey was found to be 20%, of which 19 strains (38%) were having an MIC of 15% including the standard strain of *P. aeruginosa* ATCC 27853. Honey concentrations ranging from 5% to 10% were found to be ineffective as shown

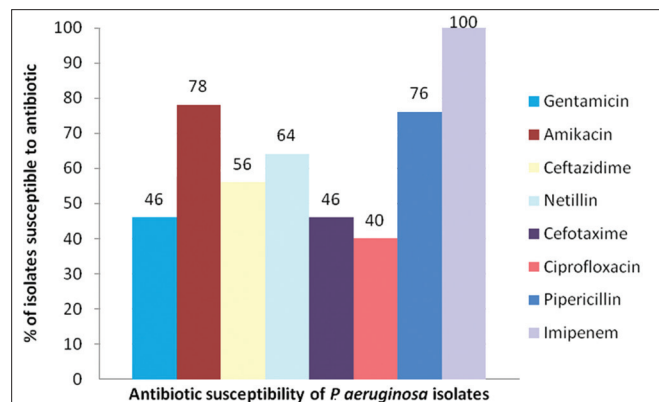


Figure 1: Percentage of susceptibility pattern to different class of antibiotics to *Pseudomonas aeruginosa* isolates used in the study

in Figure 2. The Y axis indicates the number susceptible percentage of isolates to honey and Dettol at various concentrations (5-25%) plotted on the X axis.

The time kill assay for the bactericidal effect was tested on five isolates of *P. aeruginosa* so as to understand the time required to kill the bacterial population irrespective of their antibiotic susceptibility patterns as isolates varying in their susceptibility pattern were used. Honey at concentrations of 20%, 25%, 50% could bring out complete destruction in 24 h, whereas concentration of honey at 75% and 100% could bring about complete destruction of *P. aeruginosa* in 12 h [Table 1]. The lowest concentration of the honey which showed bactericidal activity was at 20% for all the five isolates tested in time kill assay. The antibacterial activity of honey was concentration dependent for all the 50 isolates tested at a concentration below 20% using agar dilution methods [Figure 2]. The antibacterial effect of 50% honey on *P. aeruginosa* with number

of hours and percentage of survival rate was calculated. The average percentages of survival rate for five *P. aeruginosa* isolates after 4 h, 8 h, 12 h, and 24 h were found to be 22.3, 5.2, 1.1, and 0 respectively as shown in Table 2.

DISCUSSION

The present study shows the bactericidal activity of honey against *P. aeruginosa* strains. As honey is considered as a natural antiseptic in the management of wound infections, its efficacy was compared with Dettol. It was observed that these organisms were more or less susceptible to honey and Dettol at 20% and 10% irrespective to their antibiotic sensitivity patterns. Dettol antiseptic liquid contains chloroxylenol which is proved to kill a wide variety of microbes and is widely used for cleansing wound. The antibacterial action is due to disruption of the cell membrane potentials and blocking the production of adenosine triphosphate.^[17] The antibacterial effect of honey was concentration dependent and bactericidal effect was observed at concentration of 20% or more for *P. aeruginosa* isolates tested. Since *P. aeruginosa* are recalcitrant to antibiotic therapy the ability to inhibit test isolates irrespective of their antibiotic sensitivity patterns has important clinical applications.^[10] This property may make honey useful in the treatment of drug-resistant infections.

In this study, we found that Indian origin honey have activity against *P. aeruginosa* wound isolates comparable to Tualang and Manuka honey as reported in previous studies.^[18] Lusby *et al.*^[19] reported that any honey can have equivalent antibacterial activity against some bacteria compared with other pharmaceutical honeys, whereas Basson *et al.*^[20] found no such high antimicrobial activity for honeys native to South Africa. Orla *et al.*^[21] compared the activity of Ulmo90 and Manuka honey and reported similar activity against the *P. aeruginosa* at a concentration of 12.5%, better than the results from our study. Results from these studies confirm that honeys from different countries and regions may have wide variations in their antimicrobial activity. It has been shown that honey may have antimicrobial action ranging from lesser than 3% to 50% and higher concentrations.^[22] Honey has been successfully used in the treatment of surgical wounds,^[23,24] burns wound,^[6] and decubitus ulcers.^[25] It has also been shown as a good medium to store skin grafts^[26] and honey has antileishmanial effect also.^[27]

The mechanisms of antibacterial action of honey remain speculative. Honey may inhibit bacterial growth due to a number of different mechanisms. High sugar concentration, low pH, hydrogen peroxide generation, proteinaceous compounds, or other unidentified components present in

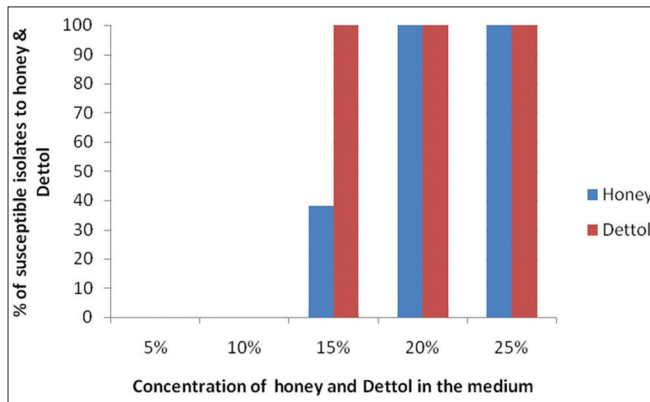


Figure 2: MIC of *Pseudomonas aeruginosa* isolates towards honey and Dettol at different concentrations

Concentration of honey	<i>Pseudomonas aeruginosa</i> (five isolates tested)
100%	12**
75%	12
50%	24
25%	24
20%	24

**Number of hours required for complete bactericidal effect

<i>Pseudomonas aeruginosa</i> clinical isolates	No. of hours and percentage of survival rates			
	4 h	8 h	12 h	24 h
Isolate 2104	22.1	5.2	1.2	0
Isolate 2099	22.4	5.1	1.0	0
Isolate 2163	22.3	5.3	1.1	0
Isolate 2897	22.4	5.3	1.0	0
Isolate 2892	22.2	5.1	1.2	0

the honey may all provide antimicrobial activity.^[28] Shrinkage and disruption of the bacteria may be due to its osmotic effect, low pH, and also due to the presence of antibacterial substance such as inhibine.^[29,30] Several components may contribute to the non-peroxide activities of honey, such as the presence of methyl syringate and methylglyoxal, which have been extensively studied in *Leptospermum* honeys.^[31] Besides its antimicrobial properties, honey can clear infection in a number of ways *in vivo*, like boosting the immune system, anti-inflammatory, and antioxidant activities and via stimulation of cell growth.^[32] After reviewing the literature it has been found that apart from Mulai *et al.*,^[33] no other Indian study has been carried out exclusively on antibacterial activity of honey against *P. aeruginosa in vitro*. As there is lack of scientific research and documentation, still the medicinal properties of Indian honeys remain in the dark. Further studies on human subjects are required *in vivo* to understand the efficacy of Indian honeys in eliminating bacteria from wounds. As this study presents the findings of *in vitro* antibacterial activity of honey against planktonic bacteria this should not be related to chronic wound environment where indeed biofilms of *P. aeruginosa* may be present and the characteristic of bacteria can change; hence, future studies in this direction will pave the way in establishing the medicinal importance of Indian honey against the sessile forms of *P. aeruginosa*.

CONCLUSION

As honey is easily available in the market and is inexpensive its antibacterial activity was comparable to other medicinal honeys. All strains of *P. aeruginosa* including both resistant phenotypes and sensitive strains were inhibited at 20% antibacterial honey concentrations *in vitro*. This intriguing observation may have important clinical implications and could lead to a new approach for treating multidrug resistant *P. aeruginosa* infected wounds using honey of Indian origin.

REFERENCES

1. Hamid S, Saeed MA. Bee keeping. Hamdard Med 1991;34:94-5.
2. Deshpande SD, Kulkarni KS. *In vitro* effect of some India honeys on *Staphylococcus aureus* from wounds. Indian J Exp Biol 2010;48:931-5.
3. Cooper RA, Molan PC, Harding KG. Antibacterial activity of honey against strains of *Staphylococcus aureus* infected wounds. J R Soc Med 1999;92:283-5.
4. Molan PC, Cooper RA. Honey and sugar as a dressing for wound and ulcers. Trop Doct 2000;30:249-50.
5. Cooper RA, Molan PC. Honey in wound care. J Wound Care 1999;8:340.
6. Subramanyam M. Topical application of honey in the treatment of burns. Br J Surg 1991;78:497-8.
7. Bhatia R, Narain JP. The growing challenge of antimicrobial resistance in the South-East Asia Region-are we losing the battle? Indian J Med Res 2010;132:482-6.
8. Molan PC. The role of honey in the management of wounds. J Wound Care 1999;8:415-8.
9. Cooper RA, Halas E, Molan PC. Efficacy of honey in inhibiting strains

of *Pseudomonas aeruginosa* form infected burns wound. J Burn Care Rehabil 2002;23:366-70.

10. Cooper RA, Molan PC. The use of honey as an antiseptic in managing *Pseudomonas infection*. J Wound Care 1999;8:161-16 4.
11. Willix DJ, Molan PC, Harfoot CG. A comparison of the sensitivity of wound infecting species of bacteria to the antibacterial activity of manuka honey and other honey. J Appl Bacteriol 1992;73:388-94.
12. Irish J, Blair S, Carter DA. The antibacterial activity of honey derived from Australian flora. PLoS One 2011;6:e18229.
13. Gethin G, Cowman S. Bacteriological changes in sloughy venous leg ulcers treated with manuka honey or hydrogel: An RCT. J Wound Care 2008;17:241-4, 246-7.
14. Baron EJ, Peterson LR, Sydney MF. Non fermentative gram negative Bacilli and coccobacilli. Bailey's and Scott's diagnostic microbiology. 9th ed. St Louis:mosby-Year Book: 1994; pp397.
15. Gopalkrishna BK. Effect of honey on bacteria isolated from burn wound infection: An *in vitro* study. Biomed 1998;18:68-72.
16. Brown R, Poxton IR. Centrifuges, colorimeters and bacterial counts. In: Collee JG, Marmion BP, Fraser AG, Simmons A, editors. Mackie and Mc Cartney Practical Medical Microbiology. 14th ed. New york: Churchill Livingstone; 1996. p. 845.
17. Aly R, Maibach HI. Comparative antibacterial efficacy of a 2-minute surgical scrub with chlorhexidine gluconate, povidone-iodine, and chloroxylenol sponge-brushes. Am J Infect Control 1988;16:173-7.
18. Tan HT, Rahman RA, Gan SH, Halim AS, Hassan SA, Sulaiman SA, *et al.* The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. BMC Complement Altern Med 2009;9:34
19. Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Res 2005;36:464-7.
20. Basson NJ, Grobler SR. Antimicrobial activity of two South African honeys produced from indigenous *Leucospermum cordifolium* and *Erica* species on selected micro-organisms. BMC Complement Altern Med 2008;8:41.
21. Orla S, Anthony D, Rahma A, Alice P, Georgina G, Seamus C, *et al.* Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. BMC Complement and Altern Med 2010;10:47.
22. Wilkinson JM, Cavanagh HM. Antibacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. J Med Food 2005;8:100-3.
23. Greenwood D. Honey for superficial wounds and ulcers. Lancet 1993;341:90-91.
24. Chiriufe J, Searmato G, Herszage L. Scientific basis for use of granulated sugar in treatment of infected wounds. Lancet 1982;1:560-1.
25. Blomfield R. Honey for decubitus ulcers. JAMA 1973;224:905.
26. Subramanyam S. Storage of skin grafts in honey. Lancet 1993;341:63-4.
27. Zeina B, Zohra BI, Al-assad S. The effects of honey on leishmania parasites: An *in vitro* study. Trop Doct 1997;27 Suppl 1:36-8.
28. Mundo MA, Padilla-Zakour OI, Worobo RW. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. Int J Food Microbiol 2004;97:1-8.
29. White JW Jr, Subers MM, Scheparts AI. The identification of inhibine, the antibacterial factor in honey as hydrogen peroxidase and its origin in a honey glucose oxidase system. Biochim Biophys Acta 1963;73:57-70.
30. Zumla A, Lulaf A. Honey-a remedy rediscovered. J R Soc Med 1989;82:384-5.
31. Mavric E, Wittmann S, Barth G, Henle T. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. Mol Nutr Foods Res 2008;52:483-9.
32. Al-Jabri AA. Honey, milk and antibiotics. Afr J Biotechnol 2005;4:1580-7.
33. Mullai V, Menon T. Antibacterial activity of honey against *Pseudomonas aeruginosa*. Indian J Pharmacol 2005;37:403.

How to cite this article: Shenoy VP, Ballal M, Shivananda PG, Bairy I. Honey as an antimicrobial agent against *Pseudomonas aeruginosa* isolated from infected wounds. J Global Infect Dis 2012;4:102-5.

Source of Support: Nil. **Conflict of Interest:** None declared.