IN VITRO ACTIVITY OF HONEY COLLECTED FROM INDIA (VELLORE) AND NEW ZEALAND AGAINST SELECTED CLINICAL PATHOGENS

Subashini Devarajan, Subhashree Venugopal*

School of Biosciences and Technology,
VIT University, Vellore - 632 014, Tamil Nadu, India.

Summary

The antibacterial activities of four honey samples collected from different locations of Vellore district and one sample from New Zealand named manuka honey were all tested against clinical pathogens such as *Staphylococcus aureus* and *Klebsiella pneumoniae*. These honey samples were compared with standard antibiotics like Ampicillin, Tetracycline, Chloramphenicol and Erythromycin. The antibacterial activity was tested using Kirby-Bauer's method for antibiotics and well diffusion method for honey samples. The honey samples were tested at concentrations of 25, 40, 50, 75% and net honey i.e. 100%. Undiluted honey samples inhibited the growth of all the strains. All diluted honey samples inhibited the growth of *Staphylococcus aureus* MTCC 737, *Staphylococcus aureus* from sputum and BAL at varying concentrations whereas, honey at higher concentrations was required to inhibit the growth of *Klebsiella pneumoniae*. None of the strains were inhibited at 25% concentration.

Key words: Honey, antibacterial activity, *Staphylococcus aureus*, *Klebsiella pneumoniae*

Correspondence to

Dr. V. Subhashree,

Assistant Professor (Senior),

School of Biosciences and Technology,

VIT University, Vellore-632014, Tamil Nadu, India.

Tel.: +91 9486947377; Fax: +91-416- 2243092 / 2240411.

E -mail: vsubhashree@vit.ac.in; v_subhashree@hotmail.com

Introduction

Development of antibiotic resistant bacteria continues to be of major health concern world-wide (1). So it is necessary to isolate active compounds from honey which can be used beyond conventional antibiotic therapy. Honey has been used since ancient times for the treatment of some respiratory diseases and for the healing of skin wounds. It has been proposed that the healing effect of honey could be due to various factors such as high osmolarity, acidity and particularly hydrogen peroxide which is formed from the oxidation of glucose by the enzyme glucose oxidase, during the period when honey is ripening (2, 3). Glucose oxidase originates from the hypopharyngeal glands of honeybees (4). When hydrogen peroxide is removed by adding catalase, some honeys still show significant antibacterial activity (5) and this activity is referred to as non-peroxide antibacterial activity. The non-peroxide factors of honeys include lysozyme, phenolic acids and flavonoids (4). All these factors give honey unique properties such as wound dressing, rapid clearance of infections, rapid suppression of inflammation, minimization of scarring and stimulation of angiogenesis as well as tissue granulation and epithelium growth (6).The floral source of honey plays an important role in its biological properties. For example, manuka honey from New Zealand is recognized for its therapeutic properties (6). Manuka honey contains several phenolic compounds, including methyl syringate and syringic acid (3, 7). By examining the antibacterial activiy against *Staphylococcus aureus*, methyl syringate was found to possess significant antibacterial activity.

Honey has also been shown to inhibit the Rubella virus in vitro (8), three species of the *Leishmania* parasite (9) and *Echinococcus* (10). Methicillin resistant and sensitive *Staphylococcus aureus* (MRSA and MSSA) are the two main strains which causes difficult to treat skin and underlying tissue infection associated with gram positive bacteria (11). Infection with *Pseudomonas aeroginosa* is the most serious infection in burn patients (12) followed by infection with *Klebsiella pneumoniae*, *E.coli*, *Staphylococcus aureus* and other pathogenic microorganisms (13). Our study is to determine the antibacterial activity of four honey samples from Vellore district and one sample from New Zealand manuka honey against clinical pathogens. All honey samples were compared with that of standard antibiotics.

Materials and Methods

Honey samples

Two honey samples (HS1, HS2) were collected from different locations of Vellore district (MV Kuppam, Pudur). These 2 samples were harvested from honey bee nests of tamarind tree and coconut tree, with the help of honey collector which is their traditional profession. Manuka honey (HS3) from *Leptospermum* sp. was purchased from Redwood trust, Christ church, New Zealand. Honey samples (HS4) and (HS5) were purchased from yelagiri hills. Honey samples were stored at 4ºC in the dark until

analyzed. For the antibacterial tests honey samples were used undiluted and at 25, 40, 50 and 75% dilutions. Antimicrobial susceptibility test were done in triplicates.

Standard drugs

A concentration of 30µg/disc of Ampicillin, Tetracycline, Chloramphenicol and Erythromycin (HIMEDIA) was employed for *S. aureus* and *K.pneumoniae*.

Bacterial strains

Two strains (S1 and S2) of *S. aureus* from sputum and BAL (Bronchia alveolar lavage), two strains of *K.pneumoniae* (S3 and S4) from sputum and BAL were obtained from St. Johns medical college, Bangalore. The clinical isolates were identified based on the standard microbiological techniques (14). These organisms were kept in nutrient broths with 50% glycerol and maintained in 3 ml plastic bottles at -70°C. Morphologically identical colonies from over night growth were picked up with an inoculating loop and suspended in 3-4 ml of nutrient broth and incubated for 2-3 hrs at 37ºC and diluted with sterile normal saline to a turbidity that matches 0.5 McFarland standard (10^6 CFU/ml), and further diluted 1: 100 in sterile nutrient broth to set an inoculum density of $1x10^4$ CFU/ml which was used for the test.

Antimicrobial Susceptibility

The antimicrobial activity of different samples of honey against different pathogens was tested using Kirby Bauer's method (15) for antibiotics and well diffusion method for honey sample. Test materials were prepared by diluting each honey sample (HS1, HS2, HS3, HS4, and HS5) in sterilized, double distilled water at different dilutions (concentration) 25%, 40%, 50%, 75% and net honey i.e. 100%. Muller Hinton Agar (MHA) plates were prepared. A loop full (4mm in diameter) of the prepared bacterial suspensions ($1x\ 10^4$CFU/ml) were separately applied to the centre of a sterile Muller Hinton agar plate and spread evenly using a sterile cotton wool. Wells were made on the inoculated plate using a sterile well borer (6mm in diameter). Then 100 micro liters of different concentrations of honey were dispensed and inoculated at 37°C for 20 hours and observed for various zones of inhibition.

Results and Discussion

Five honey samples were analyzed and the differences in average diameter of the inhibition zones (Fig 1, 2) were observed in this work. The sources of the nectar may have contributed to the differences in their antibacterial activities. The results for various activities are tabulated in Table 1 to 6. As for the antibacterial activity of various honey samples on different bacterial strains, it was observed that in *Staphylococcus aureus* from both BAL and sputum, growth was inhibited at varying concentrations, whereas in *Klebsiella pneumoniae* inhibition of growth was observed mostly at higher concentrations. Manuka honey showed activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* starting from 40% and 50% concentration respectively.

The honey samples were tested at concentrations of 25, 40, 50, 75% and 100% against *S. aureus* and *K. pneumoniae.* HS4 and HS5 honey samples showed no activity at 25% and 40% concentration for both the strains. HS1 and HS2 honey samples showed activity against *S. aureus* from sputum and BAL and *S. aureus* MTCC 737 at 50% and 40% respectively. HS1, HS4 and HS5 honey samples showed activity against *K. pneumoniae* from both the sources only at 100% concentration, whereas HS2 and HS3 showed activity at 75% and 50% respectively. Diameter of zone of inhibition increases with increase in concentration. When comparing the activity of antibiotics against honey, the maximum zone of inhibition for honey is greater when compared to the maximum value for antibiotics.

The results shown by honey samples in relation to *Staphylococcus aureus* may be important, given that in recent decades there has been a marked increase in difficulty to treat skin and underlying tissue infections associated with *S. aureus* (11). It has been informed that *S.aureus* has developed resistance to several antibiotics and that it is the principle contaminant agent in many clinical infections (16). Thus new strategies to treat wounds infected with *S.aureus* are needed, and the possibility to use honey appears as a convenient at less cost treatment opinion.

The results obtained for *Klebsiella pneumoniae* were also important, given that antibacterial resistance represents a serious problem (17) due to the permeability barrier afforded by its outer membrane. *Klebsiella pneumoniae* are opportunistic human pathogens that can be isolated from various human and clinical specimens (18), which were responsible for 7 to 10% of all associated blood stream infections in Europe, Latin America and North America, as reported by the SENTRY Antimicrobial Surveillance Program (19)

The present study showed that the honey inhibited the gram positive bacteria at lower concentration, than gram negative bacteria which requires higher concentration. Generally plant extracts are more active against gram positive bacteria than gram negative bacteria (20). Present findings were also supported by other researchers who reported that the crude powder of the galls of *Quercus infectoria* was found to be active against *S. aureus* (21). The variation of susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the honey sample. It has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally leads to leakage of ions from the cells (22). The effectiveness of honey or propolis depends on differences in chemical composition, bee species and geographical region (23). Honey inhibits the growth of dangerous bacteria such as *E.coli*, *S.aureus*, Salmonella, *Shigella* and *V.cholerae* (24). The concentration of honey might be varied in the inhibition of pathogenic organisms, thereby making honey a superior antibacterial agent compared to several known and currently prescribed antibiotics.

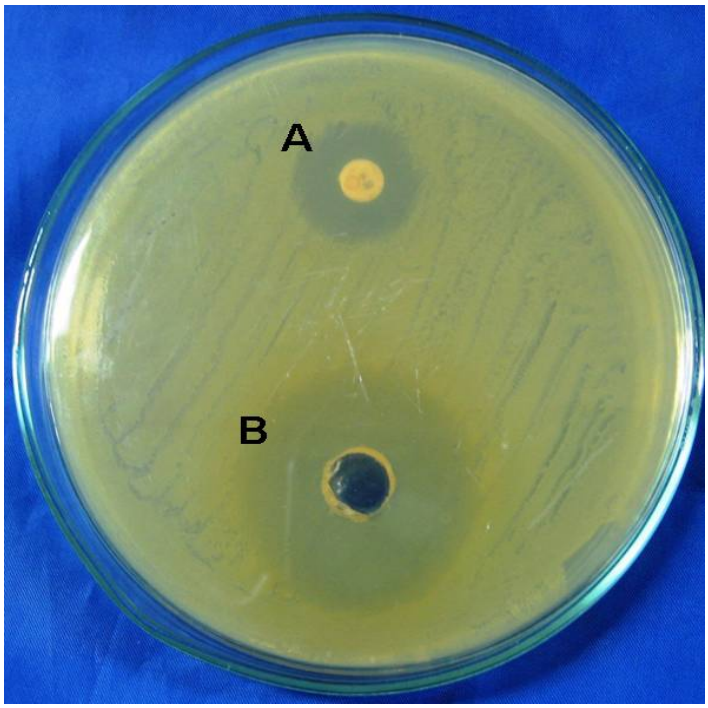

Fig.1 Antibacterial activity of coconut tree honey (HS1, 100%) against *Staphylococcus aureus*

A - Tetracycline (30µg/disc) 14 mm

B - Coconut tree honey (100%) 29mm

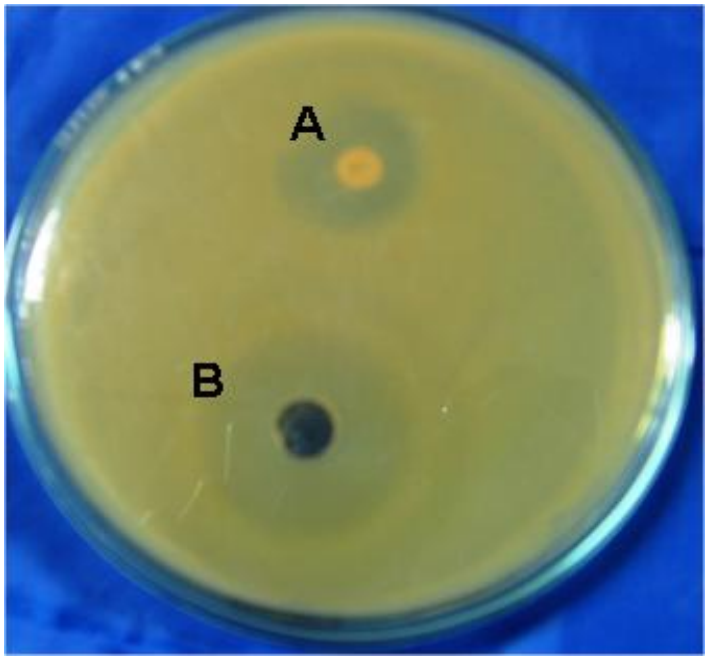

Fig. 2 Antibacterial activity of manuka tree honey (HS3, 100%) against *Klebsiella pneumoniae*

A - Tetracycline (30µg/disc) 16mm

B - Manuka tree honey (100%) 24mm

Table 1. Antibacterial Activity of Coconut tree honey (HS1) against five isolates

S.No	Clinical isolates	Sources	Honey Concentration				
			Diameter of zone of inhibition (mm)				
			25%	40%	50%	75%	100%
1	*S. aureus*	Sputum	-	-	16	25	29
2	*S. aureus*	BAL	-	-	18	27	27
3	*K. pneumoniae*	Sputum	-	-	-	-	17
4	*K. pneumoniae*	BAL	-	-	-	-	15
5	*S .aureus* MTCC 737		-	14	19	24	27

[Note: - represents no zone of inhibition]

Table 2. Antibacterial Activity of tamarind tree honey (HS2) against five isolates

S.No	Clinical isolates	Sources	Honey Concentration				
			Diameter of zone of inhibition (mm)				
			25%	40%	50%	75%	100%
1	*S. aureus*	Sputum	-	-	23	25	28
2	*S. aureus*	BAL	-	-	23	27	27.5
3	*K. pneumoniae*	Sputum	-	-	-	16	18
4	*K. pneumoniae*	BAL	-	-	-	17	19.5
5	*S .aureus* MTCC 737		-	17	22	25	26

[Note: - represents no zone of inhibition]

Table 3. Antibacterial Activity of manuka honey (HS3) against five isolates

S.No	Clinical isolates	Sources	Honey Concentration				
			Diameter of zone of inhibition (mm)				
			25%	40%	50%	75%	100%
1	*S. aureus*	Sputum	-	19	22	27	29
2	*S. aureus*	BAL	-	17	23	26	30
3	*K. pneumoniae*	Sputum	-	-	17	19	24
4	*K. pneumoniae*	BAL	-	-	16	17	25
5	*S .aureus* MTCC 737		-	24	24	27	28

[Note: - represents no zone of inhibition]

Table 4. Antibacterial Activity of Yelagiri honey (HS4) against five isolates

S.No	Clinical isolates	Sources	Honey Concentration				
			Diameter of zone of inhibition (mm)				
			25%	40%	50%	75%	100%
1	*S. aureus*	Sputum	-	-	-	19	24
2	*S. aureus*	BAL	-	-	-	18	27
3	*K. pneumoniae*	Sputum	-	-	-	-	17
4	*K. pneumoniae*	BAL	-	-	-	-	15
5	*S. aureus* MTCC 737		-	-	16	25	26

[Note: - represents no zone of inhibition]

Table 5. Antibacterial Activity of Yelagiri honey (HS5) against five isolates

S.No	Clinical isolates	Sources	Honey Concentration				
			Diameter of zone of inhibition (mm)				
			25%	40%	50%	75%	100%
1	*S. aureus*	Sputum	-	-	15	17	23
2	*S. aureus*	BAL	-	-	-	16	24
3	*K. pneumoniae*	Sputum	-	-	-	-	19
4	*K. pneumoniae*	BAL	-	-	-	-	17
5	*S. aureus* MTCC 737		-	-	17	26	27

[Note: - represents no zone of inhibition]

Table 6. Antibacterial Activity of Standard Antibiotics against five isolates

S.No	Clinical isolates	Sources	Antibiotics (30 µg/disc)			
			Diameter of zone of inhibition (mm)			
			Amp	Tet	Chl	Ery
1	*S. aureus*	Sputum	21	14	16	20
2	*S. aureus*	BAL	10	19	10	22
3	*K. pneumoniae*	Sputum	-	13	12	13
4	*K. pneumoniae*	BAL	-	16	15	14
5	*S. aureus* MTCC 737		19	15	12	17

[Note: - represents no zone of inhibition]

Conclusion

The present study demonstrated that in vitro, all the five samples of honey (HS1, HS2, HS3, HS4, HS5) had an antibacterial activity against *S.aureus* and *K.pneumoniae* obtained from the source of sputum and BAL (Bronchia alveolar lavage). The honey samples were compared with that of standard antibiotics Ampicillin, Tetracycline, Chloramphenicol and Erythromycin. Over use of antibiotics leads to side affects and also a major factor for the emergence of multidrug resistant microorganisms. So honey can be used as an excellent alternate to combat the further spread of multidrug resistant microorganisms. Further research is necessary to isolate the active compounds from these honey samples and to check the antibacterial activity. However, pharmacological standardization and clinical evaluation on the effect of honey and its active components are essential before using it as a preventive and curative measure to common diseases related to the tested bacterial species. The wider availability of honey in rural areas provides its utilization for certain diseases.

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References

1. Hseuh P, Chen W, Luh K. Relationships between antimicrobial use and antimicrobial resistance in Gram-negative bacteria causing nosocomial infections from 1991-2003 at a university hospital in Taiwan. Int. J. Antimicrob. Agents 2005; 26: 463-472.

2. Bogdanov S. Nature and origin of the antibacterial substances in honey. Lebensm-Wiss Technol 1997; 30: 748-753.

3. Weston RJ, Mitchell KR, Allen KL. Antimicrobial phenolic components of New Zealand manuka honey. Food Chem 1999; 64: 295-301.

4. Taormina PJ, Niemira BA, Beuchat LR. Inhibitory activity of honey against food borne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. Int. J. Food Microbiol 2001; 69: 217-225.

5. Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol 1991; 43: 817-822.

6. Molan P. Not all honeys are the same for wound healing. Bull Eur. Tissue Rep. Soc 2002; 9: 5-6.

7. Russell KM, Molan PC, Wilkins AL, Holland PT. Identification of some antibacterial constituents of New Zealand manuka honey. J Agri Food Chem 1990; 38: 10-13.

8. Fahey JW, Stephenson KK. Pinocembrin from honey and Thai ginger (*Boesenbergia pandurata*): A potential flavonoids inducer of mammalian phase 2 chemo protective and antioxidant enzymes. J Agri Food Chem 2002; 50: 7472-7476.

9. Zeina B, Zohra BI, Assad S. The effects of honey on Leishmania parasite: an in vitro study. Trop Doct 1997; 1: 36-38.

10. Kilicoglu B, Kismet K, Koru O. The scolicidal effects of honey. Adv Ther 2006; 23: 1077-1083.

11. Halcon L, Milkus K. *Staphylococcus aureus* and wounds: a review of tea tree oil as a promising antimicrobial. Am. J. Infect. Control 2004; 32: 402-408.

12. Altoparlak U, Aktas F, Selebi D, Ozkurt Z, Akcay M. Prevalence of metallo-β-lactamase among *Pseudomonas aeruginosa* and *Actinobacter baumanii* isolated from burn wounds and in vitro activities of antibiotic combinations against these isolates. Burns; 2005: 31: 707-710.

13. Nasser S, Mabrouk A, Maher A. Colonization of burn wounds in Ain shams university burn unit. Burns; 2003: 29:229-233.

14. Cheesbrough M. Medical laboratory manual for tropical countries. Vol II Microbiology 1998; 196-205.

15. Bauer AW, Kirby WMM, Sherirs JC, Turck M. Antibiotic susceptibility testing by standard single disk method. American Journal of Clinical Pathology 1966; 45: 433-496.

16. Moreno J, Cruz C, Renzoni A. Tracking methicillin resistant *Staphylococcus aureus* Colombian hospitals over 7 years (1996-2003): emergence of a new dominant clone. Int. J. Antimicrob Agents 2005; 25: 457-462.

17. Conceicao T, Brizio A, Duarte A, Barros R. First isolation of $bla_{\text{VIM-2}}$ in *K.oxytoca* clinical isolates from Portugal. Antimicrob. Agents Chemother 2005; 49: 476.

18. Podschun R, Ullmann U. Klebsiella spp. As nosocomial pathogens: epidemiology, taxonomy, typing methods and pathogenecity factors. Clin Microbiol Review 1998; 11:589-603.

19. Biedenbach DJ, Moet GJ, Jones RN. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from SENTRY Antimicrobial Surveillance Program. Diagn. Microbiol. Infect. Dis 2004; 50: 59-69.

20. Lin J, Opaku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK. Preliminary screening of some traditional Zulu medicinal plants for anti inflammatory and anti microbial activities. J Ethanopharmacol 1999; 68: 267-274.

21. Fatima S, Farooqi AHA, Kumar R, Kumar TRS, Khanuja SPS. Antibacterial activity possessed by medicinal plants used in tooth powders. J. Med. Aromatic plant Sci 2001; 22: 187-189.

22. Walsh SE, Maillard JY, Russel AD, Catrenich CE, Charbonneau AL, Bartolo RG. Activity and mechanism of action of selected biocidal agents on Gram positive and negative bacteria. J. Appl. Microbiol 2003; 94: 240-247.

23. Miorin PL, Levy NCJ, Custodio AR, Bretz WA, Marcucci MC. Antibacterial activity of honey and propolis from *Apis mellifera* and *Tetagonisca angustula* against *S. aureus*. J. Applied. Microb 2003; 95: 913-920.

24. Zumla A, Lulat A. Honey a remedy rediscovered. J. R. Soc. Med 1989; 82: 384-385.