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Antibacterial activity of honey against clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* serovar TyphiShyamapada Mandal^{1*}, Manisha DebMandal², Nishith Kumar Pal¹, Krishnendu Saha¹¹Department of Microbiology, Bacteriology and Serology Unit, Calcutta School of Tropical Medicine, C. R. Avenue, Kolkata–700 073, India²Department of Physiology and Biophysics, KPC Medical College and Hospital, 1F Raja S C Mallick Road, Jadavpur, Kolkata–700 032, India

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ABSTRACT

Objective: To ascertain the potential antibacterial activity of honey against clinical isolates of *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Salmonella enterica* serovar Typhi (*S. enterica* serovar Typhi) by *in vitro* methods. **Methods:** The partial inhibitory concentration (PIC), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the autoclaved honey (extracted from *Apis indica* hive by indigenous method) were determined for *S. enterica* serovar Typhi ($n=8$; from blood culture), *E. coli* ($n=5$; from urine culture) and *P. aeruginosa* ($n=5$; from pus culture) isolates by *in vitro* methods. **Results:** The PICs of the honey tested for the isolates ranged 0.50%–1.25 % (v/v) for *S. enterica* serovar Typhi, 0.75%–1.50% (v/v) for *E. coli* and 1.00%–1.25 % (v/v) for *P. aeruginosa*, while the MICs ranged 1.75%–3.00% (v/v), 3.00%–3.50% (v/v) and 3.50% (v/v), respectively. The *P. aeruginosa* and *E. coli* isolates had MBC value of 4.00% (v/v); the *S. enterica* serovar Typhi showed MBCs in between 3.00% and 3.50% (v/v). The bactericidal activity of honey was achieved at concentration 3.00% (v/v) for *S. enterica* serovar Typhi and *E. coli*, and at 3.50% (v/v) for *P. aeruginosa*. **Conclusions:** The excellent antibacterial activity of honey against clinical bacterial isolates indicates the usefulness of honey in clinical practice against bacterial infection.

1. Introduction

The continuous use of antibiotics in clinical practice has been the direct cause of the development of multiple antibiotic resistances among bacteria causing human infection[1]. To combat such bacterial resistance to antibiotic, scientists discovered natural sources like medicinal plants of non-antibiotic drugs having antibacterial potentiality[2–4]. Beside the medicinal plants, the antibacterial activity of honey against many different life threatening bacteria has been reported[5–8]. Wilkinson [9] determined the activity of 13 different honey samples, including three commercial antibacterial honeys, against *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). It has been reported that honey showed both bacteriostatic and bactericidal effect against gram positive as well as gram-negative bacteria, and also exhibited anti-fungal activity[10,11]. Chauhan *et al*[12]

reported that the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of honey ranged 0.625–5.000 mg/mL for the clinical isolates of *E. coli*, *P. aeruginosa* and *Salmonella enterica* serovar Typhi (*S. enterica* serovar Typhi). Another study revealed honey MIC 11% for *Pseudomonas* isolates[13]. Moreover, honey represents the oldest traditional medicines in the treatment of respiratory ailment, gastrointestinal infection and various other diseases. It is being used effectively as a dressing for wounds (including surgical wounds), burns, and skin ulcers to reduce pain and odour quickly. Molan[14] documented an array of supportive evidences ranging from case reports to randomized controlled trials mentioning the value of honey in wound care, particularly its antibacterial activity. Honey has been reported to maintain moist wound environment that promotes healing, and its high viscosity helps to provide a protective barrier to prevent infection; in addition, the mild acidity and low-level hydrogen peroxide release help in tissue repairing and contribute to the antibacterial activity[15]. However, from our part of the country no report has been documented based upon the scientific study on antibacterial activity of honey. Herein, we report the *in vitro* antibacterial activity of honey produced by honeybees (*Apis*

*Corresponding author: Dr. Shyamapada Mandal, Department of Zoology, Gurudas College, Narkeldanga, Kolkata–700 054, India.
E-mail: samtropmed@gmail.com.

indica) (*A. indica*) against clinical isolates of *E. coli*, *P. aeruginosa* and *S. enterica* serovar Typhi.

2. Materials and method

2.1. Bacterial strains and media

A total of 18 bacterial isolates that included *E. coli* ($n=5$), *P. aeruginosa* ($n=5$) and *S. enterica* serovar Typhi ($n=8$), obtained respectively from urinary tract infection cases by urine cultures, human skin lesion by pus cultures and suspected enteric fever patients by blood cultures, at the Calcutta School of Tropical Medicine, Kolkata, India were assessed. The *E. coli* ATCC 25922 strain was used as the control. Urine, pus and blood samples from suspected patients were cultured using McConkey agar, blood agar and brain heart infusion broth, respectively, for the recovery of *E. coli*, *P. aeruginosa* and *S. enterica* serovar Typhi.

2.2. Processing of honey for antibacterial activity

Honey sample, harvested from *A. indica* hive during spring 2007, from a village of district Purulia, West Bengal (India) in sterile screwed cups was used in the study. The honey sample was filtered through a sterile cheese cloth to remove debris, autoclaved at 121 °C for 15 min, streaked on blood agar and nutrient agar plates in duplicate, and incubated for 24 h at 35 °C to check microbial purity. The pH of the honey was checked and stored at 4 °C until used.

2.3. Antibacterial activity

Antimicrobial activity of honey for the bacterial isolates has been determined by agar dilution method^[16]. Molten nutrient agar (Hi-Media, Mumbai, India) was distributed (20 mL each) in 12 sterile culture tubes and autoclaved at 121 °C for 15 min. The tubes containing media were held in water bath (55 °C) to add honey at different concentrations (50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700 and 800 μ L), which were equivalent to honey concentrations 0.25%, 0.50%, 0.75%, 1.00%, 1.25%, 1.50%, 1.75%, 2.00%, 2.50%, 3.00%, 3.50% and 4.00% (v/v), respectively. The media from the tubes were plated to obtain 12 culture plates each containing different concentrations of honey as mentioned above. The plates thus prepared were divided into 18 equal sectors for spot inoculation of the test microorganisms. After inoculation, with 10^4 CFU/spot, the plates were incubated for 24 h at 35 °C. The nutrient agar plate without honey was similarly inoculated to control the appropriate growth of the organisms. Results were noted in terms of bacterial growth on the agar plates.

2.4. Interpretation of results

The partial inhibitory concentration (PIC) was reported as the lowest concentration of honey that retarded growth as compared to the control plate, and the minimum inhibitory concentration (MIC) was reported as the lowest concentration of honey required for inhibiting the visible growth of the isolates. The minimum bactericidal concentration (MBC) was

determined by further sub culturing the last plate, which showed visible growth, and all the plates in which there was no growth on agar medium. The MBC was thus the lowest concentration of honey required to produce sterile culture.

2.5. In vitro killing activity

Killing activity of honey against three bacterial strains: *S. enterica* serovar Typhi D1/01, *E. coli* EC4 and *P. aeruginosa* PS1 (randomly selected) were determined by using the initial inoculum of 5×10^5 CFU/mL ($5.698 \log_{10}$ CFU/mL) of nutrient broth followed by incubation for 24 h at 35 °C. Honey concentrations used for the study ranged from 0.50% (v/v) to 5.00% (v/v). Bactericidal activity was defined as a $\geq 3 \log_{10}$ decrease in the inoculum after 24 h of incubation^[17].

3. Results

The PICs, MICs and MBCs of honey (pH 3.5) for the isolates of *S. enterica* serovar Typhi ($n=8$), *E. coli* ($n=5$) and *P. aeruginosa* ($n=5$) are represented in Figure 1. The PIC values for the isolates ranged variously: 0.50%–1.25% (v/v) for *S. enterica* serovar Typhi, 0.75%–1.50% (v/v) for *E. coli* and 1.00%–1.25% (v/v) for *P. aeruginosa*. The *P. aeruginosa* isolates showed top MIC value of honey (3.50%, v/v), while the MICs of honey ranged between 3.00% and 3.50% (v/v) for *E. coli* isolates, and from 1.75% to 3.00% (v/v) for *S. enterica* serovar Typhi isolates. The *P. aeruginosa* and *E. coli* isolates had high MBC value of 4.00% (v/v); the *S. enterica* serovar Typhi showed MBCs in between 3.00% and 3.50% (v/v).

The effect of different concentration of honey, ranging from 0.50% to 4.00% (v/v), on the growth of *S. enterica* serovar Typhi, *P. aeruginosa* and *E. coli* is represented in Figure 2. The bacterial strains grew well in presence of 0.50% (v/v). The honey started to show growth inhibitory effect at concentration 1.00% (v/v) for *S. enterica* serovar Typhi and *E. coli*, and 1.50% (v/v) for *P. aeruginosa*. Bactericidal activity of honey was achieved at concentration 3.00% (v/v) for *S. enterica* serovar Typhi and *E. coli*, and at 3.50% (v/v) for *P. aeruginosa*. Growth of *S. enterica* serovar Typhi, *E. coli* and *P. aeruginosa* was completely inhibited beyond concentrations 3.00%, 3.50% and 4.00% (v/v) honey, respectively.

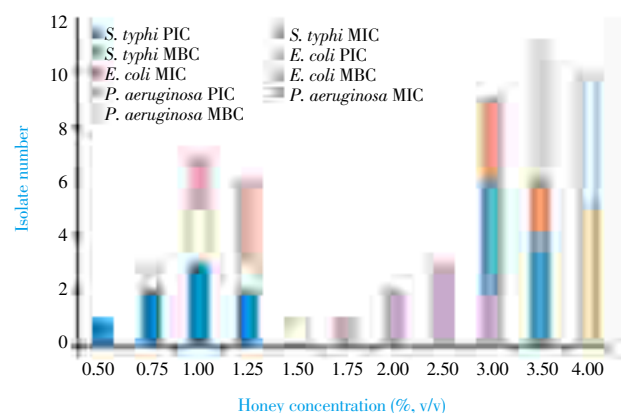


Figure 1. PIC, MIC and MBC of honey for *S. enterica* serovar Typhi (*S. typhi*), *E. coli* and *P. aeruginosa* isolates.

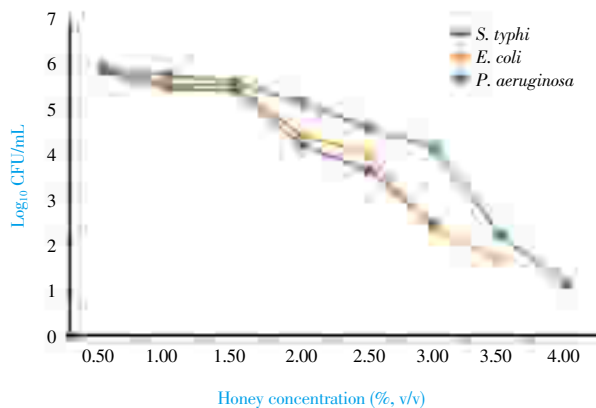


Figure 2. Effect of different concentration of honey on the growth of *S. enterica* serovar Typhi (*S. typhi*), *E. coli* and *P. aeruginosa* isolates.

4. Discussion

It has been reported that the honey produced by honeybees (*A. mellifera*) inhibited most of the test organisms at concentrations 2.5%–7.5% (v/v)[18], while this value was 1.75%–3.50% in the present communication. Chauhan *et al*[12] reported that the most susceptible bacteria, in a study with honey, included *Salmonella typhi*, *E. coli* and *P. aeruginosa* having MICs and MBCs of honey in the range of 0.625–5.000 mg/mL, and ZDI for the isolates ranged 6.94–37.94 mm, respectively. Mulu *et al*[18] reported that the tualang honey had more PIC (a lower concentration that retarded growth) than the manuka honey. When tested against *S. enterica* serovar Typhi and *P. aeruginosa*, tualang honey and manuka honey had equal MICs (15% and 17.5%, respectively), while the values were 17.5%–22.5% for *E. coli*[5]; in the same study, the MBCs were recorded as 17.5%–20%, 22.5%–25% and 17.5%–25%, respectively, for *S. enterica* serovar Typhi and *P. aeruginosa* and *E. coli*, while the ZDIs for the bacterial isolates were 24 mm for tualang honey and 26 mm for manuka honey. Asadi-Pooya *et al*[19] documented that the growth of mycobacteria was inhibited at honey concentrations of 10% and 20% but not at 5%, 2.5% or 1% concentration. Agbagwa and Frank-Peterside[20] reported ZDI in between 3 mm and 17 mm for four different pathogenic bacterial genera due to honey action from different parts of Nigera. The honey sample, in the present study, exhibited PIC (bacteriostatic) at concentrations 0.50%–1.25%, and bactericidal activities for all the test microorganisms at concentrations 3.00%–4.00% (v/v), based on the agar dilution techniques. When *in vitro* time–kill study was considered, the honey showed bactericidal activity at 3.00% (v/v) for *S. enterica* serovar Typhi and *E. coli*, and at 3.50% (v/v) for *P. aeruginosa*. The ≥ 3 log₁₀ decrease in CFU/mL (3.264 log₁₀, 3.378 log₁₀, and 3.508 log₁₀ CFU/mL decrease, respectively, for *S. enterica* serovar Typhi, *E. coli*, and *P. aeruginosa*), compared to the initial inocula of 5.698 log₁₀ CFU/mL, after 24 h incubation supported the phenomena.

The current study showed that honey has less antimicrobial activity against *P. aeruginosa* as compared with the other test microorganisms, *S. enterica* serovar Typhi and *E. coli*.

The high PICs (1.00%–1.25%) and MIC (3.50%) of honey for all the isolates of *P. aeruginosa* supported this view too. The wide range of MICs of different honeys against the same class of microorganisms has been reported illustrating differences in antibacterial potency of different honeys[15]. The average MIC and MBC of honey for test bacterial isolates were recorded as 6.2% and 8% (v/v), respectively[21]. Among different honeys tested, the Khadikraft honey was found best with 11% MIC; the other types of honeys had MIC of 20% against *P. aeruginosa*[22]. The antimicrobial effect of honey samples against *S. aureus* and *S. epidermidis* was found different that in turn indicated difference in the sensitivity of these bacteria to the antimicrobial activity of honey[23]. Thus, the above fact underlines the value of using a standardized medical grade honey that demonstrates consistent antibacterial activity against a broad range of microorganisms.

The honey produced by honeybees (*A. mellifera*) showed both bacteriostatic and bactericidal activity when tested *in vitro*, as has been reported by Mulu *et al*[18]. Honey, at 60% concentration, was found bacteriocidal for *P. aeruginosa* and bacteriostatic for *S. aureus* and *Klebsiella* sp[24]. The findings of the present study are in resonance with the above. The variation in the antimicrobial potency of honey has been reported too. The concentration of honey for full prevention of growth of *E. coli*, as has been reported by Mulu *et al*[18] was 6.5%, and for *P. aeruginosa* the value was 7.5%. Basson *et al*[10] demonstrated that the honey concentration needed for complete growth inhibition of *S. anginosus* and *S. oralis* were 17% and 12.5%, respectively. Growth retardation and complete inhibition have been observed at concentrations 2.5% and 6% (v/v), respectively, as has been reported by Mulu *et al*[21]. French *et al*[25] reported that the growth of Staphylococcus isolates was inhibited by manuka and pasture honeys at concentrations 2.7–5% (v/v), whereas the simulated honey inhibited the test isolates at concentrations 27.5–31.7% (v/v), showing 5.5–11.7 times greater antibacterial activity of natural honeys; such activity was due to the osmotic effect of the sugar content of honey. In the current study, the values were recorded as 3.00%–3.50% for *E. coli* isolates, and 3.50% for *P. aeruginosa* isolates. The *S. enterica* serovar Typhi isolates in our study showed lower PICs (0.50%–1.25%) and MICs (1.75%–3.00%) of honey.

The variation in the antimicrobial potential of honey used in the present study as compared to the others might be due to differences in growth rate of pathogens, inoculum size and the test method it self, as well as source of the microorganisms. Tan *et al*[5] stated that honey is produced from many sources, and its antimicrobial activity varies greatly with origin and processing. Also, it might be the fact that the type of honey produced by honeybees is dependent on the natural vegetative flowers blooming in different seasons and in different places, and thus the flowers from which bees gathered nectar to produce the honey may contribute to the difference in the antimicrobial activities of honey[18]. Several earlier authors reported that the antimicrobial activities have been attributed to its high acidic nature (pH being 3.2–4.5), high osmotic effect, hydrogen peroxide concentration and its phytochemical nature; beside this, it has been reported that methylglyoxal, which is present in high concentration in manuka honey,

is directly responsible for its characteristic antibacterial property^[26–28]. In the present study, we used autoclaved honey that showed excellent antibacterial activity *in vitro*, which suggests that the antibacterial activity of honey is not dependent alone on its phytochemical nature, *i.e.* tetracycline derivatives, ascorbic acid, peroxidase or amylases, streptomycin, sulfonamides, which are reported as heat labile. We checked the pH of the honey as 3.5. The honey when added to the media lowers the pH of the media up to 6 to 6.5, which might inhibit the bacterial growth with other factors, in the media.

In conclusion, the honey showed excellent antibacterial activity against *E. coli*, *P. aeruginosa* and *S. enterica* serovar Typhi, related respectively to the urinary tract infection, skin lesion and enteric fever among human patients, and thus the honey may be considered against such common infection. The antimicrobial properties as a topical agent has been described and documented both in *in vitro* and *in vivo* studies and evidence supports its usefulness in wound healing^[29]. However, further studies include pharmacological standardization and clinical evaluation on the effect of honey in order to consider it (honey) as a preventive and curative measure to the infection caused by the test bacterial strains.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Mandal S, Deb Mandal M, Pal NK. Antimicrobial resistance pattern of *Salmonella typhi* isolates in Kolkata, India during 1991–2001: A retrospective study. *Jpn J Infect Dis* 2002; **55**: 58–9.
- [2] Mandal S, Deb Mandal M, Pal NK. Synergistic anti-*Staphylococcus aureus* activity of amoxicillin in combination with *Embllica officinalis* and *Nymphae odorata* extracts. *Asian Pac J Trop Med* 2010; **3**: 711–4.
- [3] Kumar P, Prasad R, Chandra H, Bhatt RP, Sati OP. *In vitro* antibacterial activity of *Juniperus communis* L. against bacterial pathogens. *Environ Conserv J* 2009; **10**: 101–4.
- [4] Amin M, Kalantar E, Mohammad-Saeid N, Ahsan B. Antibacterial effect and physicochemical properties of essential oil of *Zataria multiflora* Boiss. *Asian Pac J Trop Med* 2010; **3**: 439–42.
- [5] 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.
- [6] Kingsley A. The use of honey in treatment of infected wounds: case studies. *Br J Nursing* 2001; **10**: S13–20.
- [7] Ghori I, Ahmad SS. Antibacterial activities of honey, sandal oil and black pepper. *Pak J Bot* 2009; **41**: 461–6.
- [8] Chambers J. Topical manuka honey for MRSA-contaminated skin ulcers. *Palliat Med* 2006; **20**: 557.
- [9] Wilkinson JM. Antibacterial Activity of 13 Honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. *J Med Food* 2005; **8**: 100–3.
- [10] 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.
- [11] Nasir NM, Halim AS, Singh KB, Dorai AA, Haneef MM. Antibacterial properties of tualang honey and its effect in burn wound management: a comparative study. *BMC Complement Altern Med* 2010; **10**: 31.
- [12] Chauhan A, Pandey V, Chacko KM, Khandal RK. Antibacterial activity of raw and processed honey. *Electronic J Biol* 2010; **5**: 58–66.
- [13] Mullai V, Menon T. Antibacterial activity of honey against *Pseudomonas aeruginosa*. *Indian J Pharmacol* 2005; **57**: 403.
- [14] Molan PC. The evidence supporting the use of honey as a wound dressing. *Int J Low Extrem Wounds* 2006; **5**: 40–54.
- [15] Lusby P, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. *Arch Med Res* 2005; **36**: 464–7.
- [16] National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically*. Approved standard. 4th ed. M7–A4. Wayne, PA: National Committee for Clinical Laboratory Standards; 1997.
- [17] Leclercq R, Bingen E, Su QH, Lambert-Zechovski N, Courvalin P, Duval J. Effects of combinations of β -lactams, daptomycin, gentamycin and glycopeptides against glycopeptide resistant enterococci. *Antimicrob Agents Chemther* 1991; **35**: 92–8.
- [18] Mulu A, Tessema B, Derbie F. *In vitro* assessment of the antimicrobial potential of honey on common human pathogens. *Ethiop J Health Dev* 2004; **18**: 107–12.
- [19] Asadi-Pooya AA, Prnjehihahin MR, Beheshti S. The antimycobacterial effect of honey: an *in vitro* study. *Biol Forum* 2003; **96**: 491–6.
- [20] Agbagwa OE, Frank-Peterside N. Effect of raw commercial honeys from Nigeria on selected pathogenic bacteria. *Afr J Microbiol Res* 2010; **4**: 1801–3.
- [21] Mulu A, Kassu A, Tessema B. Antibacterial activity of honey produced by honeybees (*Apis mellifera*) on bacterial species isolated from infected wound. *Ethiopian Pharmaceutical Journal* 2005; **23**: 1–6.
- [22] Mullai V, Menon T. Bactericidal activity of different types of honey against clinical and environmental isolates of *Pseudomonas aeruginosa*. *J Alternative Complementary Med* 2007; **13**: 439–41.
- [23] Baltrusaityte V, Venskutonis PR, Eksteryte V. Antibacterial activity of honey and beebread of different origin against *S. aureus* and *S. epidermidis*. *Biotechnol* 2007; **45**: 201–8.
- [24] Noori S. Al-Waili. Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva. *J Med Food* 2004; **7**: 210–22.
- [25] French VM, Cooper RA, Molan PC. The antibacterial activity of honey against coagulase-negative *Staphylococci*. *J Antimicrob Chemother* 2005; **56**: 228–31.
- [26] 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.
- [27] Adams CJ, Boulton CH, Deadman BJ, Farr JM, Grainger MN, Manley-Harris M, et al. Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr Res* 2008; **343**: 651–9.
- [28] Atrott J, Henle T. Methylglyoxal in manuka honey—correlation with antibacterial properties. *Czech J Food Sci* 2009; **27**: S163–5.
- [29] Cooper RA, Halas E, Molan PC. The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. *J Burn Care Rehabil* 2002; **23**: 366–70.