INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ANTIBACTERIAL POTENTIAL OF MANUKA HONEY BV 20+ JOINT AGAINST RESISTANT *SALMONELLA ENTERICA SEROVAR TYPHI* CLINICAL ISOLATES

Syed Zohaib Hussain [1]*, Sarah Hussain [2], Zafar Ali Seenharo [3], Muhammad Bilal [4], Samreen Mujahid [5]
[1] College of Pharmacy, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan
[2] Dow University of Medical and Health Sciences, Karachi, Pakistan
[3] Department of Forensic Medicine, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan
[4] College of Pharmacy, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan
[5] Baqai Medical University Karachi, Pakistan
*Corresponding Author Email: zohaib.hussain@lumhs.edu.pk

Article Received on: 27/09/19 Approved for publication: 02/11/19

DOI: 10.7897/2230-8407.1011314

ABSTRACT

Antibiotic resistance poses a major task in managing typhoid associated with *Salmonella enterica* serovar *Typhi* (*S. Typhi*). Multidrug-resistant (MDR) isolates of *Salmonella* are prevalent in regions of Asia. Especially in areas having informal settlements with improper sanitation and clean water supply. The prime object of the study was to identify and determine the antibacterial potential of Manuka honey (MH) BV20$^+$ joint undiluted and at different dilutions against resistant strains of *S. Typhi. S. Typhi* clinical isolates (n = 30*)* were collected from Civil Hospital Karachi, Pakistan. Antimicrobial potential of Manuka honey (MH) BV20$^+$ joint and the sensitivity pattern of pathogen were detected by using Agar well diffusion method. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were also calculated by using micro-broth dilution technique. From thirty clinical isolates, no one showed resistance against undiluted manuka honey as well as at 4000 µL/mL dilution. No samples showed resistance against azithromycin, used as positive control. The average zone of inhibition of azithromycin against *S. Typhi* was 27.11 mm while that of undiluted manuka honey was 25.28 mm. MIC and MBC were found to be 4000 µL/mL. Manuka honey BV20+ joint showed good antibacterial potential against *Salmonella enterica serovar* Typhi indicating its significance in clinical practice as an empirical therapy. Further investigation is required to evaluate role of manuka honey as complementary and alternative medicine against *Salmonella enterica serovar Typhi* infection.

Key words: Manuka Honey, sensitivity pattern, clinical isolates, potential, clinical practice.

INTRODUCTION

Antimicrobial resistance has steadily been increased against antibiotics[1]. Resistance shown by bacteria is not only an alarming issue but also leads to remarkable degrees of problem[2]. Additional mutations enhance the survival of resistant bacteria[3]. In order to maintain the efficiency of existing antimicrobials it is imperious to decrease pattern of antimicrobial resistance[4]. *Salmonella enterica serovar Typhi* induced typhoid fever is one of the intimidating disease[5]. Asian regions including Pakistan China, Vietnam, and India are more prone to *Salmonella enterica* with nearly 80% fatalities[6,7]. Antibiotics resistance developed by *S. typhi* is due to adaptability, genetic diversity and chromosomal mutations[8]. Resistance has been increased to quinolones in various regions especially in Asia[9]. For centuries, honey has been used because of its beneficial effects against treatment of various diseases. Currently, various honeys have been marketed with standard label of antibacterial activity, but Manuka honey produced from *Leptospermum scoparium* is well known for its antibacterial activity[10]. *In vitro* antimicrobial properties of Manuka honey of New Zealand origin were reported against some pathogenic bacteria[11]. Researchers have shown efficiency of *L. scoparium* origin Manuka honey, against human pathogens including *S. Typhi*[12,13]. Honey including Manuka Honey (MH) has antibacterial action against gram-negative and gram- positive bacterial pathogens[14].

MATERIALS AND METHOD

Collection of clinical isolates

Salmonella enteric clinical isolates (n = 30) were collected from blood samples of typhoid patients using standard sterile measures at Civil Hospital Karachi, Pakistan.

Isolation and identification of organism

Isolation of clinical isolates was done at Civil Hospital on the basis of morphology and biochemical reactions, API 20E strips test and triple sugar iron tests were also performed for confirmation.

Collection of honey sample

Manuka Honey BV20$^+$ joint was purchased from USA by Calcomp Nutrition Inc. bearing specimen no. MHWGBV250-1

Preparation of honey dilutions

Dilutions of honey were prepared with distilled water to the required concentrations i.e. 2000 µL/ml, 4000 µL/ml and 6000 µL/ml(v/v). All dilution samples were incubated in shaking water bath for solution aeration up to 30 min at temperature 37°C. Both

H_2O_2 and glucose are light sensitive, so incubation was performed in dark[15].

Collection of Antibiotic

Azithromycin 500 mg (Zithromax, Pfizer species) was purchased.

Susceptibility testing

Sensitivity and resistance pattern were determined[16].

Preparation of inoculum, broth and media plates

To evaluate sensitivity and resistance pattern of clinical isolates of *Salmonella enterica serovar Typhi*, Muller-Hinton (Oxoid Ltd, England) medium was used. Only colonies with same morphological type were selected. National Committee for Clinical Laboratory Standards (NCCLS) guidelines were used to prepare Mueller-Hinton broth and agar medium. Surface of each colony was taken by the sterile wire loop and transferred to test tube containing broth (4-5 ml). Broth was incubated for 8-24 hours at 37°C. Bacterial suspension with suitable turbidity was prepared and 0.5 McFarland standards (McS) were used as a reference[17]. Sterile cotton swab dipped in bacterial suspension streaked over the Mueller-Hinton agar surface in three directions to obtain growth uniformity and finally plates were left for 10 minutes.

Application of material in well

Wells having diameter of 6-8 mm were made in culture media with the help of sterile cork borer under aseptic conditions, Manuka honey undiluted (1 ml) along with dilutions of 2000 µL/ ml ,4000 µL/ ml and 6000 µL/ml(v/v) were applied in punched wells by sterile syringe (3 mL). Azithromycin 15 μg was used as control[18].

Incubation of plates

Plates were then incubated for 24 hours at 37°C. Vernier caliper was used to measure the diameter of the zones of growth inhibition around individual wells.

Minimum inhibitory concentration (MIC)

Micro-broth dilution method was performed for determination of MIC. Dilutions of Manuka honey in distilled water along with concentrations i.e. 1000, 2000, 3000, 4000 and 5000 µL/ml were prepared. Mueller–Hinton broth (2 ml) and honey (2 ml) were mixed. 1 ml of standardized inoculums having 3.3 x 106 CFU/ml was added to each test tube and incubated in aerobic condition at temperature 35°C for 24 hours. Broth and honey containing tubes lacking inoculum served as positive control while broth and inoculum containing tubes as negative control. Test tubes were analyzed after 24 hours incubation period in order to evaluate minimum inhibitory concentration. Absence of growth at lowest concentration showed MIC[19,20].

Minimum Bactericidal Concentration (MBC)

Sterile Mueller-Hinton agar plates were independently inoculated with test tubes in which no traces of growth were seen. Those plates were re-incubated in incubator at 35°C for 24 hours and then examined. The highest dilution with no bacterial growth was MBC[19,20].

RESULT

Manuka honey undiluted was proven to be very effective against all tested clinical isolates of *S. Typhi* even at 4000 µL/mL dilution. From thirty clinical isolates, no one showed resistance against undiluted manuka honey as well as at 4000 µL/mL dilution. The average zone of inhibition of Azithromycin against *S. Typhi* was 27.11 mm. None of the isolates showed resistance against azithromycin while the average zone of inhibition of manuka honey BV20+ joint against *S. Typhi* was found to be 25.28 mm. The zone of inhibition values is given in Table 1. MIC and MBC were found to be 4000 µL/mL. MIC and MBC values are provided in Table 2.

Table 1: Antibacterial activity of Manuka honey BV20+ joint

Isolates	Average zone of Inhibition (mm)				
	Test				Control
	Honey (ml)	Honey dilutions (µL / mL)			Azithromycin Control
	Undiluted	2000	4000	6000	mL
Salmonella enteric serovar Typhi	25.28 mm	18.11 mm	22.37 mm	25.21 mm	27.11 mm

NOTE: (NZ; No Zone of inhibition)

Table 2: MIC and MBC of different dilutions of Manuka honey BV20+ joint

Isolates	MICµL					MBCµL				
	1000	2000	3000	4000	5000	1000	2000	3000	4000	5000
Salmonella enterica serovar Typhi	D	D	SD	ND	ND	D	D	SD	ND	ND

NOTE (MIC; Minimum Inhibitory Concentration, MBC; Minimum Bactericidal Concentration, ND; Not Detected, D; Detected, SD; Slightly Detected)

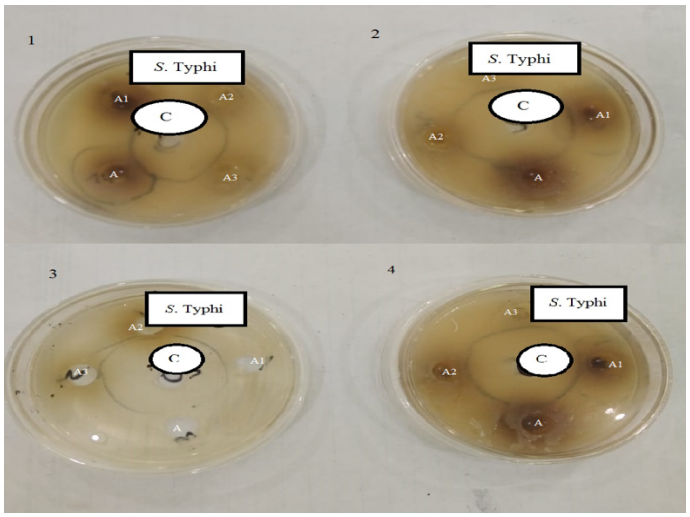

Figure 1(A) Manuka honey Undiluted, (A1) dilution 6000 µL/ml, (A2) dilution 4000 µL/ml, (A3) dilution 2000 µL/ml
2. (A) Manuka honey Undiluted, (A1) dilution 6000 µL/ml, (A2) dilution 4000 µL/ml, (A3) dilution 2000 µL/ml
3. (A) Manuka honey Undiluted, (A1) dilution 6000 µL/ml, (A2) dilution 4000 µL/ml, (A3) dilution 2000 µL/ml
4.(A) Manuka honey Undiluted, (A1) dilution 6000 µL/ml, (A2) dilution 4000 µL/ml, (A3) dilution 2000 µL/ml

DISCUSSION

In Asian countries including Pakistan, typhoid fever is the major health problem. Its etiology is directly related to health care set-up and cleanness. S. *Typhi* resistance against antibiotics is considered as chief public problem. The purpose of the study was to determine antibacterial activity of Manuka honey BV20+ joint undiluted and at different dilutions against Salmonella enterica serovar Typhi clinical isolates. Numerous literatures have reported antimicrobial activities of honey against various microorganisms[10]. 80% w/v Manuka honey has been shown to disrupt *Salmonella typhi* bio film[25]. Antimicrobial activity of Manuka honey against *Staphylococcus spp., E. coli* and *Salmonella spp*. has been reported[26]. Antimicrobial activity of Manuka honey against numerous bacterial strains has already been proved[21,22]. Manuka honey in some cases showed poor activity[23]. However, New Zealand's manuka honey showed good antibacterial properties[24]. During current study, manuka honey was proven to be active against *Salmonella enterica* serovar Typhi clinical isolates. Manuka honey showed excellent zones of inhibition as comparable to Azithromycin used as control. From thirty clinical isolates, no one showed resistance against undiluted manuka honey as well as at 4000 µL/mL dilution. Manuka honey at 4000 µL/mL dilution was also active and zone of inhibition obtained was 22.37 mm indicating effectiveness of manuka honey $BV20^+$ joint even at dilution. This may be due to the fact that Manuka honey's antibacterial activity is more powerful as compared to conventional honey's regular peroxide activity because of presence of Unique Manuka factor in it[27].

CONCLUSION

Manuka honey $BV20^+$ joint showed good antibacterial potential against *Salmonella enterica serovar Typhi* reflecting its importance in clinical practiceas an empirical therapy. Further investigation is required to evaluate role of manuka honey as complementary and alternative medicine against *Salmonella enterica serovar Typhi* infection to overcome the alarming situation of resistance development in *S. Typhi*.

ACKNOWLEDGEMENT

I am very thankful to Prof. Dr. Baqir S. Naqvi, Department of Pharmaceutics, Hamdard University, Karachi, Pakistan for his valuable support during work.

REFERENCES

1. Colpan A, Porter S, Johnston B, Clabots C, Anway R, Thao L, Kuskowski MA, Tchesnokova V, Sokurenko EV, Johnson JR. *Escherichia coli* sequence type 131 (ST131) as an emergent multidrug-resistant pathogen among U.S. veterans. Clin. Infect. Dis 2013; 57: 1256–1265.
2. Felmingham D, Cantón R, Jenkins SG. Regional trends in beta-lactam, macrolide, fluoroquinolone and telithromycin resistance among *Streptococcus pneumoniae* isolates 2001-2004. J Infect 2007; 55(2): 111-118.
3. Personal author: Esposito JL. The obsolete self: Philosophical dimensions of aging. Los Angeles: University of California Press; 1987.
4. Sharma R, Sharma CL, Kapoor B. Antibacterial resistance: Current problems and possible solutions. Ind J of Med Sci 2005; 59(3): 120-129.
5. Das A, Seenivasan SH, Umachandran S, Arumugam G, Magudeshwaran K. Molecular characterization of *Salmonella enterica Serovar Typhi* isolated from typhoidial humans. Malays J Microbiol 2012; 8: 148-155.
6. Ling J, Chang PY. Plasmid-mediating resistance to chloramphenicol, trimethoprim and ampicillin in *Salmonella typhi* in the Southeast Asian region. J Infect Dis 1984; 149: 652.
7. Mermin JH, Villar R, Carpenter J. A massive epidemic of multidrug resistant typhoid fever in Tajikistan associated with consumption of municipal water. J Infect Dis 1999; 179: 1416-1422.
8. Winstaniey C, Hart CA. Secretin systems and pathogenicity islands. J Med Microbiol 2001; 413: 848-852.
9. Xia S, Hendriksen RS, Xie Z, Huang L, Zhang J. Molecular characterization and antimicrobial susceptibility of *Salmonella* isolates from infections in Humans in Henan Province, China. J Clin Microbiol 2009; 47: 401-409.
10. Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol 1991; 43: 817-822.

11. Sherlock O, Dolan A, Athman R, Power A, Gethin G, Cowman S., Humphreys H. 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 Altern Med 2010; 10: 47
12. Visavadia BG, Honeysett J, Danford MH. Manuka honey dressing: An effective treatment for chronic wound infections. Br J Maxillofac Surg 2006; 44: 38–41.
13. Saranraj P, Sivasakthi S, Feliciano GD. Pharmacology of honey-a review. Adv Biol Res 2016; 10: 271-89.
14. Lusby PE, Coombes AL, Wilkinson JM. Bactericidal Activity of different honeys against pathogenic bacteria. Arch. Med. Res 2005; 36(5): 464-467.
15. Haley RW, Culver DH, Morgan WM, White JW, Emori TG, Hooton TM. Identifying patients at high risk of surgical wound infection. A simple, multivariate index of patient susceptibility and wound contamination. Am J Epidemiol 1985; 121: 206–15.
16. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement; CLSI Document M100-S18; Clinical Laboratory Standards Institute: Wayne, PA, USA; 2014.
17. Masood H, Naqvi SB, Aslam N. Cost effective analysis of different brands of ceftriaxone available in Karachi Pakistan. Pak J Pharmacol 2008; 25: 13-19.
18. Chauhan A, Pandey V, Chacko KM, Khandal RK. Antibacterial activity of raw and processed honey. Electronic J Biol 2010; 5: 58-66.
19. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complement. Altern. Med 2005; 5: 6.
20. Yushau M, Onourah FC, Murtala Y. *In-vitro* sensitivity pattern of some urinary tract isolated to *Carica papaya* extracts. Bay. J Pur. and App. Sci 2009; 2: 75–78.
21. Wilkinson JM, Cavanagh HM. Antibacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. J Med Food 2005; 8: 100-103.
22. French VM, Cooper RA, Molan PC. The antibacterial activity of honey against coagulase-negative *Staphylococci*. J Antimicrob Chemother 2005; 56: 228-231.
23. Basson NJ, du Toit IJ, Grobler SR. Antibacterial action of honey on oral *Streptococci*. J Dent Assoc S Afr 1994; 49: 339-341.
24. Molan PC. The antibacterial properties of honey. Chemistry in New Zealand; 2004. p. 10-14.
25. Hannan A, Bajwa AE, Riaz S, Arshad U, Saleem S, Bajwa UI. *In vitro Salmonella typhi* bio film formation on gallstones and its disruption by manuka honey. Pak. J. Pharm. Sci 2018; 31(1): 129–135.
26. Johnston M, Mc BrideM, Dahiya D, Owusu R, Nigam P. Antibacterial activity of Manuka honey and its components: An overview. AIMS Microbiol 2018; 4: 655-664.
27. Georgescu MI, Dobrea, M, Dobrea VC. Antimicrobial effect of commercial manuka honey and conventional local honey against gram-negative and gram-positive bacteria. Sci Work. Ser C. Vet Med 2018; 12 (2): 133-136.

Cite this article as:

Syed Zohaib Hussain *et al*. Antibacterial potential of Manuka Honey BV 20+ joint against resistant *Salmonella enterica Serovar typhi* clinical isolates. Int. Res. J. Pharm. 2019;10(11):20-23 http://dx.doi.org/10.7897/2230-8407.1011314

Source of support: Nil, Conflict of interest: None Declared